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UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 727

Contribution from the Bureau of Plant Industry
WM. A. TAYLOR, Chief

Washington, D. C. PROFESSIONAL PAPER December 18, 1918

ANTHRACNOSE OF CUCURBITS

By

M. W. GARDNER, Scientific Assistant
Cotton, Truck, and Forage Crop Disease Investigations

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SCOPE OF THE INVESTIGATION.

Anthracnose of cucurbits is a common and widely distributed disease of the cucumber, muskmelon, watermelon, certain gourds, and a few other cucurbits. It is apparently limited to this family of host plants.

The disease is caused by the fungus *Colletotrichum lagenarium* (Pass.) Ell. and Hals., and is characterized by sunken or discolored lesions on the leaves, stems, and fruits of its host plants. These lesions are not limited in size, and under certain conditions the

[1] The data here presented were obtained as the result of a cooperative arrangement between the Bureau of Plant Industry, the University of Wisconsin, and certain cucumber growers during the seasons of 1915, 1916, and 1917. The writer, as a graduate student, was designated by the University of Wisconsin to carry out its part in the cooperation, which also extended to Indiana and Michigan under the leadership of W. W. Gilbert, of the Bureau of Plant Industry. Most of the laboratory studies herein reported were conducted in the laboratory of plant pathology of the University of Wisconsin, and the article itself was presented to the faculty of that university in partial fulfillment of the requirements for the degree of doctor of philosophy.

The writer wishes to acknowledge his indebtedness to Prof. L. R. Jones, of the University of Wisconsin, for supervision and helpful suggestions throughout the progress of this work, and to Dr. W. A. Orton, Mr W. W. Gilbert, and Dr. E. Carsner for their assistance and cooperation.

production of new lesions is very rapid. Therefore, upon its hosts under cultivation this disease often becomes epiphytotic and may cause serious loss. While primarily a field-crop disease it also occurs as a greenhouse trouble in cucumber culture.

Anthracnose was noted as early as 1867, and it now occurs quite commonly throughout Europe and the eastern United States. The disease has received considerable attention from mycologists and plant pathologists, and while diverse names were given to the causal organism agreement seems to have been reached that the several descriptions apply to the same fungus.

Among the outstanding disputed questions are that of the correct generic name of the fungus and that of the relation of this fungus to the causal organism of bean anthracnose. The latter question is about settled.

As in the case of other anthracnoses, the increased prevalence of this disease following wet weather has been recognized. It seems to be rather generally held that this anthracnose may be controlled by spraying.

In the present bulletin it has been the purpose to bring together and summarize the work of others upon this disease and to add something from observation and experiment. While a little is added to the record in the way of a description of the disease, the main purpose has been to learn more details regarding the life history of the causal fungus with relation to the disease and to devise a method of control with special reference to the disease as it occurs in the cucumber-pickle crop. The phases studied in particular are the overwintering of the parasite, manner of introduction into fields, mode of dissemination, method of host infection, and means of control.

THE DISEASE.

HOSTS.

The economically important hosts of anthracnose are the cucumber (*Cucumis sativus*), the muskmelon (*Cucumis melo*), and the watermelon (*Citrullus vulgaris*). Among the noneconomic hosts are gourds of the genus Lagenaria, two species of Cucumis, *Benincasa cerifera*, and *Trichosanthes colubrina*. Saccardo (42, v. 3, p. 719–720)[1] lists *Cucumis colocynthis* as a host.

While Farlow (17, p. 202) in his host index includes squash and pumpkin as hosts, subsequent observations indicate that anthracnose does not occur as a vine disease in the genus Cucurbita, although it is reported on squash fruits (45, p. 15). More details regarding the host range of the fungus are presented later, in the consideration of pathogenicity.

[1] The serial numbers in parentheses refer to "Literature cited," at the end of this bulletin.

HISTORY.

The first authentic report of anthracnose is that of Passerini (16), who in 1867 found the disease on Lagenaria fruits at Padua, Italy. Eight years later, he (16) reported its extensive occurrence on watermelons and cantaloupes in the Province of Parma, Italy.

In 1871 and in 1876 Berkeley (3, 4) reported in the Gardener's Chronicle a Gloeosporium on cucumber fruits in England, the identity of which is doubtful. He (4) noted that Gloeosporia occurred on many hosts and thought them all cross inoculable. During the latter year D. T. Fish (18), a cucumber grower, reported in the same journal a new disease in his greenhouses which he recognized as distinct from the well-known downy mildew. From his rather careful description of the symptoms and behavior of this disease on cucumbers and muskmelons it seems quite likely that it was anthracnose.

Roumeguère (41), in France, published in 1880 a rather detailed account of this disease, occasioned by its occurrence in epidemic form on melons at Chalons the year previous. In a letter to Roumeguère in 1880 Saccardo reports the disease as causing serious damage in Italy since 1877.

In Germany Frank (19, p. 518) reported in 1883 that a Gloeosporium had been destructive on cucumbers and melons. Acting upon Berkeley's suggestion he tried cross inoculations of cucumber fruits with bean anthracnose. Negative results led him to conclude that the two species of fungi were distinct.

In America the chief interest seems to have centered about the relation of the bean and the cucumber anthracnoses. The disease was noted as early as 1885 on gourds in Philadelphia by Dr. Eckfeldt and on watermelons in Wisconsin by Prof. A. B. Seymour, according to specimens listed by Ellis and Everhart (15, p. 112; 23).

Cavara (8, p. 179), at Pavia, Italy, in 1889 found the fungus parasitic upon the stems and first leaves of *Lagenaria vulgaris* in the botanical garden. He noted that the plants were killed by the disease and that its spread was very rapid. Later, in 1892, he (39) reported the disease on the cotyledons, foliage, stems, and fruits of different cucurbits in the gardens of Pavia.

Galloway (20) in 1889 reported anthracnose on melons in New Jersey, Virginia, and North Carolina. Halsted (23), in New Jersey, reported a serious blight of cucumbers in 1890 and of muskmelons in 1892 due to this disease. Basing his belief upon successful cross inoculation from a watermelon fruit to bean pods and from both of these to a citron fruit, he concluded that the fungus was identical with that of bean anthracnose.

Although not recognized as the same disease previously studied by Roumeguère, anthracnose of melons was described by Prillieux and Delacroix (39) in France in 1894. They noted that young plants

were killed and that fruits of older plants did not mature. Studies were made upon the pathological anatomy. Attempts at control with flowers of sulphur and Bordeaux mixture were unsuccessful.

Between 1894 and 1902 spraying experiments were conducted in New Jersey by Halsted. Success was obtained with Bordeaux and soda Bordeaux mixtures (37, p. 11). Stewart (47, p. 159) in 1897 records much damage to cucumbers in New Jersey by anthracnose. During the years 1896, 1897, and 1898 Selby (43, 44) studied the disease in Ohio, where great damage was done to muskmelons and to cucumbers grown for pickles. He noted that showers and heavy dews favored the disease and found that a Bordeaux spray checked its spread among muskmelons. In 1894 Garman, of Kentucky (22, p. 51), noted anthracnose on melons shipped from the Gulf States and in 1901 gave a brief description of the disease, in the course of which he warns against seed infection.

Tubeuf in his textbook (53, p. 486–487) states that this fungus is very injurious to seedlings of watermelon, muskmelon, and gourd. He lays emphasis upon the injury to cotyledons and stems. In 1904 Eckardt (11) in Germany recommended soaking cucumber seed for one hour in ammoniacal copper carbonate in order to prevent the spread of anthracnose.

During the years 1901 and 1903 cucumber diseases seem to have become increasingly prevalent in this country. Although downy mildew was probably the main source of loss, anthracnose received added attention, and the more or less purely mycological work of the past began to be supplemented by work of a pathological nature.

Stone and Smith (49, p. 64) in 1902 reported that anthracnose had been prevalent on muskmelons and watermelons in Massachusetts in 1899 and that it was also common among greenhouse cucumbers in 1901. In 1903 Stone (48, p. 35) again briefly described the disease and noted that it occurred early in the season in greenhouses. Clinton, in Connecticut (10, p. 246), reported in 1904 that anthracnose was a common and widespread trouble among cucumbers and muskmelons, which recurred annually. The same year, Chester and Smith (9, p. 28) published the results of unsuccessful cross inoculations from bean to cucurbits made at Cornell University. These led them to conclude that bean anthracnose is due to a separate and distinct fungus.

In the same year Sheldon (46, p. 127–137), of West Virginia, published the results of the most complete study of this disease that had been made up to that time. Anthracnose was the cause of serious damage to watermelons and muskmelons in that State. He described the symptoms of the disease, and the morphology of the fungus and made numerous cross inoculations. Among his interesting observations was the fact that anthracnose was more severe on land

previously cropped to melons. He recognized the danger of dissemination by the transportation of diseased fruit and by means of contaminated seed. As a result of spray tests with watermelons he found soda Bordeaux to be a successful control, while normal Bordeaux mixture and ammoniacal copper carbonate were ineffective.

Between 1903 and 1908 this disease received considerable attention from Orton, of the United States Department of Agriculture. He (31, p. 553) noted that in 1903 anthracnose was more prevalent in cucumbers from New Jersey to Connecticut than in 1902, and his annual observations during subsequent years reveal its prevalence, as follows:

In 1904 (32, p. 584) anthracnose was relatively unimportant except upon watermelons in South Carolina, West Virginia, and elsewhere. In 1905 (33, p. 607) it was common on cantaloupes and caused losses of 50 to 100 per cent of the crop in Nebraska. On cucumbers it was more prevalent than in the previous year. It caused losses in North Carolina, West Virginia, Ohio, and Massachusetts, and together with wilt caused a loss of $100,000 in Nebraska. A severe epidemic occurred on watermelons in West Virginia.

In 1906 (34, p. 503) the disease was prevalent on cantaloupes in Indiana, Nebraska, New Jersey, and West Virginia and on cucumbers in New Jersey, North Carolina, West Virginia, Nebraska, Wisconsin, and Ohio. In Ohio a loss of 25 to 60 per cent of the crop was estimated. Anthracnose of watermelon was reported from Nebraska, Ohio, Indiana, Rhode Island, and South Carolina, and an epidemic occurred in the Ohio Valley, especially in West Virginia.

In 1907 (35, p. 582) anthracnose of cantaloupes caused injury in Massachusetts and Vermont and was reported from New Jersey on cucumbers. Anthracnose was prevalent in the region around Norfolk, Va., and was also reported from Massachusetts, New Hampshire, Nebraska, New Jersey, North Carolina, and Vermont. Anthracnose of watermelons was recorded from New Jersey, South Carolina, and Ohio, and another epidemic occurred in the Ohio Valley.

In the 1908 report (36, p. 534), anthracnose is recorded as the cause of foliage injury to watermelons in the South and the statement is made that the disease had been gaining headway during the few years previous.

This increasing importance of anthracnose as well as the recurrent downy mildew led to rather extensive attempts at control by spraying. Besides the trials made by Selby and by Sheldon, Orton and Garrison published in 1905 (37) the results of spraying tests with cucumbers and melons in South Carolina. The results of these tests seem rather inconclusive.

Potebnia (38, p. 82), of Russia, published in 1910 the result of rather extensive studies upon the causal fungus of this disease, including inoculation experiments. Krüger, in a general consideration of Gloeosporia in 1913, reported an unsuccessful attempt to infect cucumber fruits with the fungus of bean anthracnose (28, p. 294) and concluded that there is no reason for considering the fungi identical (28, p. 311). Matouschek (29) in 1914 recorded a heavy attack on cucumbers at Vienna, and since no bean anthracnose occurred near by as a source of infection he held that the two fungi were

not identical. Traverso (52) reported a severe attack of anthracnose near Chioggia, Italy, in 1913, which lowered the value of a large part of the crop.

Working at the University of Wisconsin in 1913 and 1914, Carsner[1] found that anthracnose caused damping-off of cucumber and muskmelon seedlings when the seed was immersed in a spore suspension before planting. He secured damping-off due to anthracnose in cases where seed was planted in soil collected from diseased fields four months previously, and likewise in sterilized soil with which were incorporated dry, chopped, diseased vines collected five months before. In the case of seeds dipped in a spore suspension, he found that formaldehyde treatment prevented subsequent seedling infection.

In 1915 Eriksson (16, p. 121–125) reported at length on certain difficulties with anthracnose on cucumbers and melons in Sweden. The occurrence of the disease from 1910 to 1913 among garden and greenhouse cucumbers is recorded. In one locality it seems to have caused a total loss of the greenhouse crops in 1912 and 1913 and to have discouraged cucumber and melon culture. Very convincing evidence is presented to show that the disease was introduced with seed of a much-prized Rockford variety from England, and Eriksson makes the very important suggestion that clean seed be used.

Taubenhaus (51) in 1916 reported the serious prevalence of the disease in Delaware on watermelons, cucumbers, cantaloupes, citrons, and cultivated gourds, and he reports cross inoculations proving the identity of the fungus on all of these hosts.

From this review of the history of the disease it is seen that much of the earlier work is naturally of a purely mycological nature, although some of the early writers appreciated fully the serious nature of anthracnose. Later, with the adoption of the phytopathological point of view, interest centered about the pathogenic characteristics of the fungus and the control of the disease.

GEOGRAPHIC DISTRIBUTION.

Anthracnose of cucurbits apparently occurs wherever the hosts are grown in a humid climate. This includes Europe and the United States east of the Rocky Mountains. Except for a report from Arizona in 1912 and another from Colorado in 1917 anthracnose has not been reported from the arid regions of the West.

The following data relative to the occurrence and distribution of the disease in this country since 1908 were obtained from the records of the Office of the Plant Disease Survey of the United States Department of Agriculture. The blanks in the earlier records are no doubt due in part to incomplete reports. (Table I.)

[1] Carsner, E. Studies upon cucumber diseases. MS. thesis, University of Wisconsin, 1914. (Not published.)

TABLE I.—*The distribution of anthracnose of cucurbits in the United States during the years 1908 to 1915, inclusive.*

[Abbreviations: C = on cucumbers, M = on muskmelons, W = on watermelons.]

State.	1908	1909	1910	1911	1912	1913	1914	1915	1916
Alabama			W		W	W			
Arizona					W				
Arkansas								M	
Connecticut		M		M C					
Delaware								M C W	M C W
Georgia							W		W
Illinois				C					
Indiana	M	M W	M W	M W	M W	M W	M W	M C W	C
Iowa					W		M W	W	W
Kansas								W	
Louisiana						W			
Massachusetts	C	C W	C W	C	C			C	C
Maryland							M	C	
Michigan					C	C		C	C
Minnesota					M			C	
Missouri									W
New Jersey	M C W	M C W					W	W	M
North Carolina			W		M C W	W?		W	W
New York							M	M C	
Ohio	M C	C	C	M C	M	M C W	M C	M C W	M C W
Pennsylvania							W	W	
South Carolina			M W			W		W	W
Texas								W	
Virginia			C W	M W		W		M C W	
West Virginia	W	C W				W	M	C W	
Wisconsin							C W	C	M C

Certain observations relative to the incidence of this disease are of interest here. In survey trips made by the writer in 1915 anthracnose was found in only 1 among 23 cucumber fields visited near Wautoma, Wild Rose, and Almond, Wis., in none of the 10 fields visited near Portage, and in only 1 of the 13 fields visited at Baraboo. On the other hand the disease was found in 3 among the 10 fields visited at Neshkoro, 2 out of 6 at Princeton, and 17 out of 21 near Sparta. Thus the disease seemed to prevail in certain localities and not in others.

In rather extensive surveys made in the region around Ripon, Wis., in 1914 and again in 1916, Mr. Carsner was unable to find this disease although it was abundant around Princeton, 16 miles distant.

In 1916 the writer was unable to find this disease in 12 cucumber fields visited near Baraboo, but found it in 9 out of 11 fields near Princeton. With regard to these two localities, the situation remained much as in 1915.

In the neighborhood of Madison, anthracnose was found in 1916 in only 1 out of 39 garden patches of cucumbers and in none of the 8 private fields of muskmelons. In 7 gardens visited at Burnett, Wis., no anthracnose was found on cucumbers.

In the southern melon region in 1917 the disease was found quite prevalent among watermelons, but in 16 fields of muskmelons visited near Blackville, S. C., anthracnose was found in only one field, and in that case it had very evidently come from a row of badly dis-

eased cucumber plants adjacent to the melons. No anthracnose was found in about 80 acres of pickling cucumbers inspected in Alabama.

Therefore, much remains to be explained concerning the distribution of this disease. Whether or not soil differences have anything to do with the occurrence of anthracnose is at present unknown. However, it is worth while to point out that the Princeton-Neshkoro and the Sparta regions of Wisconsin are characterized by very light sand, while the Portage and Baraboo regions have heavier soils. Evidence which proves that the fungus overwinters in the field will be presented later.

ECONOMIC IMPORTANCE.

As has been previously recorded, anthracnose has caused very serious losses from time to time among its host crops in almost every part of its range. In Europe the epidemics in Italy, France, and Sweden and in this country the repeated outbreaks of the disease throughout the Atlantic Coast States, the Ohio Valley, and Nebraska have been mentioned.

Orton (33, p. 607) reports that in 1905 from 50 to 100 per cent of the cantaloupe crop was lost in Nebraska, while in 1906 (34, p. 503) a loss of between 25 and 60 per cent of the cucumber crop in Ohio occurred. It will be recalled that Eriksson (16, p. 121) reports the disease so serious on cucumbers under glass as to discourage that industry in one locality in Sweden.

Among the estimates of loss recorded in the Office of the Plant-Disease Survey are the following:

Anthracnose of muskmelon caused a 35 per cent loss in Indiana in 1908; considerable fruit rot in Connecticut in 1909; 5 per cent crop injury in two counties in Minnesota in 1912; and a large percentage of the crop injured in Arkansas and a 25 per cent crop injury in southeastern Virginia in 1915. Anthracnose of cucumbers caused a 50 per cent crop injury, with a loss of $1,000,000, in southern Michigan in 1912 and a 25 per cent crop injury in Norfolk County, Va., in 1915. Anthracnose of watermelon caused a 50 per cent crop injury in two counties in Virginia in 1910; 75 per cent loss in places in West Virginia in 1913; 15 per cent injury in Delaware, 5 per cent in North Carolina, and 25 per cent in Norfolk County, Va., in 1915; 10 per cent injury in Georgia and 30 per cent injury in South Carolina in 1916.

High temperature and humidity are favorable to the disease; hence its ravages are worse where these conditions prevail. This may explain its destructiveness on cucumbers grown under glass. In this respect the disease has been very important in Massachusetts. Among field cucumbers grown for slicing purposes the disease may cause loss either by vine injury or by fruit disfiguration. Among coldframe cucumbers, where the individual plants represent a greater value, anthracnose may cause great loss by its attack on the foliage, especially where overhead watering is practiced. One grower near Norfolk claimed a loss of at least $1,000 in 1917 on 1½ acres, due to vine injury by anthracnose alone.

In the cucumber crop grown for pickling purposes, anthracnose may become epiphytotic, especially in warm wet seasons, and may result in serious loss due to foliage injury and consequent reduction in yield, as well as to occasional direct loss from fruit infection. The latter development is rather uncommon and is noted as a rule only on the larger dill stock and on pickles that have remained too long in transit.

To illustrate the severity and importance of the anthracnose of cucumbers, the results of disease surveys made in Wisconsin, Michigan, and Indiana in 1915 and 1916 are herewith presented.

In Wisconsin, the disease usually assumes the proportions of an epiphytotic only late in the season and is not therefore as serious as some of the other diseases. In 1915 out of 84 fields anthracnose was found in 24 and was causing serious loss in 5. In 1916, anthracnose was found in 1 field out of 12 visited near Baraboo and in 10 out of 11 examined near Princeton. As previously noted, the disease seems to be much worse in certain localities than in others.

In Michigan in 1915, anthracnose was reported from 6 fields out of over 35 inspected and was very serious in 2 fields. In 1916 the disease was not at all prevalent and was noted in only 4 fields out of about 30 visited.

In Indiana in 1915, anthracnose was considered second only to mosaic in importance and was reported from 18 fields in 9 localities. It was the cause of serious injury in 9 fields. In 1916 the disease was found in 64 fields in 18 localities and was causing serious damage in 20 fields. Anthracnose was considered the most serious foliage disease of cucumbers in northern Indiana during 1915 and 1916.

Among muskmelons, the attack of this disease on the vines seems o be more severe than in the case of cucumbers, and the factor of ıruit injury may assume great importance. Outbreaks of the disease on this crop in 1906 and 1907 have been noted. In 1917 the disease did not seem to be at all prevalent in the commercial muskmelon fields of the South.

Probably the attack on the watermelon crop is more severe and more universal than is the case with the other economic hosts. The annually recurrent epiphytotics in the Ohio Valley have been noted. The spotting of the fruits is a familiar sight in our northern markets. The potential importance of the disease is, of course, very great in a crop in which the individual fruits represent so much value and are so long exposed to infection in the field. Besides the disfiguration of mature fruits, which may be followed by rotting, there is a large loss in the field, due to reduction in yield by vine injury and by attack on the immature fruit. Since a vine does not usually mature more than two melons, any injury to young fruits is a very important factor.

In the southern melon districts in 1917 anthracnose was in general so retarded by drought that in most fields the attack came too late to cause serious loss. Exceptional fields were found, such as one 120-acre field near Quitman, Ga., in which it was evident that the yield would be reduced by several carloads, a loss of, perhaps,

$500. At Live Oak, Fla., there was a report of a total loss of the crop on one 15-acre field in 1916 due to this disease.

To summarize, it is evident that this disease under certain conditions becomes epiphytotic and causes serious losses, especially among watermelons and coldframe cucumbers. On the pickle crop it is of less importance. Anthracnose seems to be primarily a disease of large fields rather than small gardens, a disease which, as a rule, becomes a serious factor only where its hosts are grown in extensive "pure culture."

DESCRIPTION.

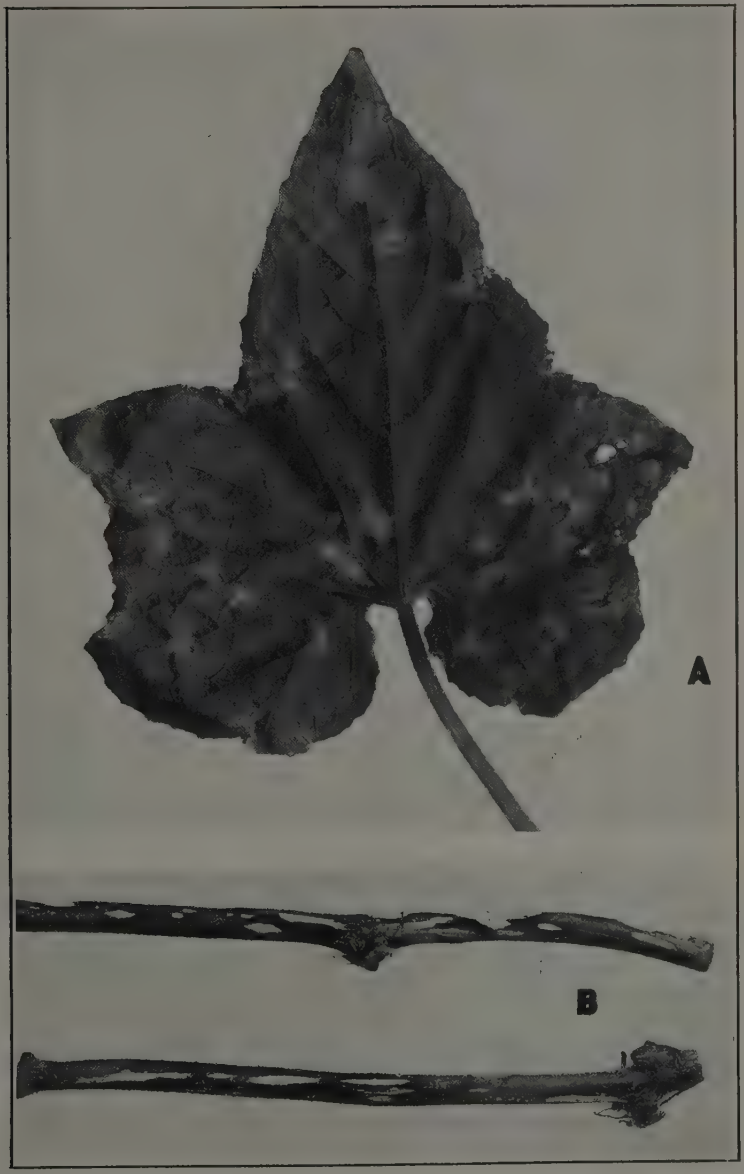
The symptoms of this disease vary somewhat with the different hosts. All parts of the host plant are subject to attack. Lesions tend to increase continuously in size. Acervuli are formed in abundance.

On cucumber leaves the lesions usually first appear on a vein and become angular or amœboid in shape, owing to the more rapid development and consequent rhizoid extension along the veins. The lesion consists of reddish brown, dry, dead tissue, often surrounded in its earlier stages by a narrow, yellowish, translucent or water-soaked border (Pl. I, *A*). Inconspicuous acervuli are produced along the veins on the upper epidermis of the brown center. Larger lesions become more circular and blotchlike (Pl. II, *B*). The dead centers crack transversely and may be beaten out by rain. Leaves being killed by the coalescence of a few large lesions become ragged in appearance (Pl. II, *B*). Very numerous incipient lesions may cause the sudden blighting of leaves. On young rapidly growing leaves small lesions may cause crinkling and extreme distortion.

Cucumber petiole and stem lesions are linear to narrowly oval, first slightly sunken and water-soaked or yellowish. Numerous acervuli are formed. The surface of such lesions later becomes quite dry and chalky in appearance (Pl. I, *B*). Cucumber stem lesions tend to remain shallow and superficial, and the collapse of mature stems at diseased points is uncommon.

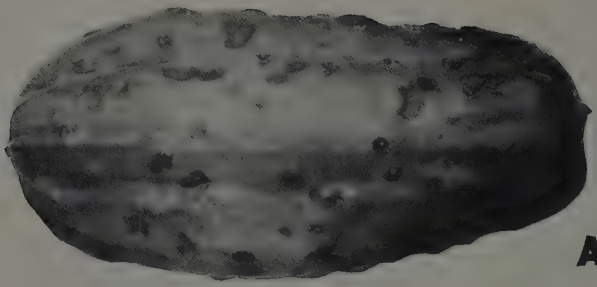
Cucumber fruit lesions appear first as more or less circular, sunken, water-soaked areas. Sporulation is abundant. The surface of such lesions becomes buff or pinkish in color, later turning to black. On mature fruits the black lesions may show white central areas bearing the old acervuli as conspicuous black dots. A dry rot is usually produced in the underlying tissue and the sunken epidermis may crack, exposing a cavity underneath.

In the field, anthracnose of cucumbers is characterized by the parched or scorched appearance and spotting of the leaves. The chief damage is due to the destruction of the leaf laminæ. Stem and petiole attack is not very evident and fruit injury is conspicuous only in the seed crop.



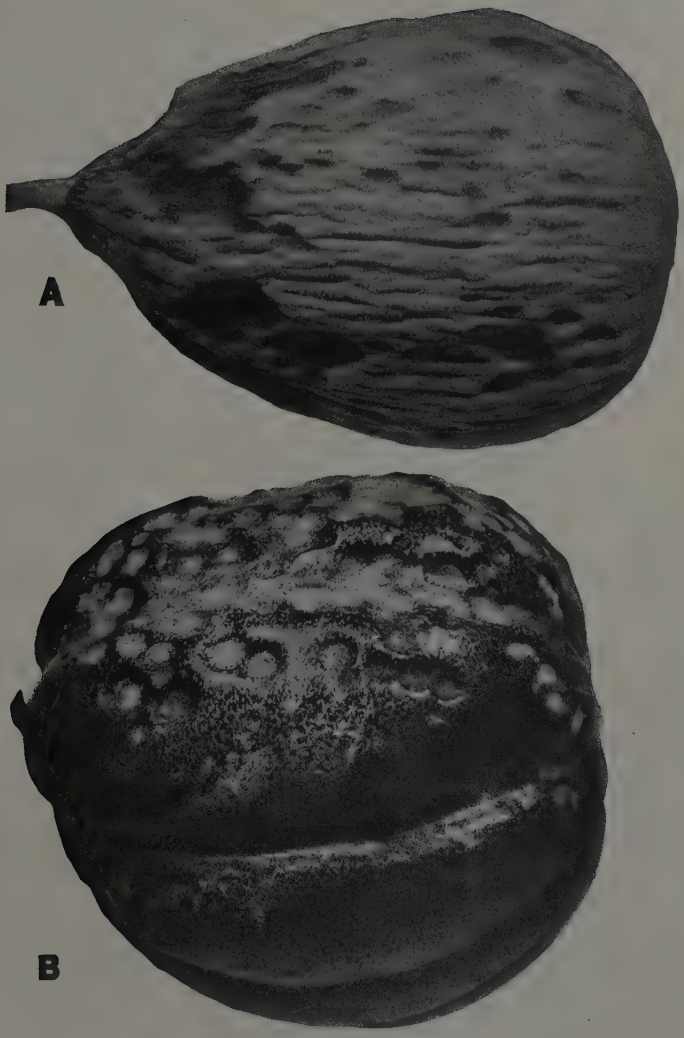
ANTHRACNOSE LESIONS ON A LEAF AND STEMS OF CUCUMBER.

A, Young lesions on a leaf, Madison, Wis., August 19, 1916; *B*, conspicuous whitened stem lesions, Madison, Wis., August 19, 1916.



ANTHRACNOSE LESIONS ON A FRUIT AND LEAF OF CUCUMBER.

A, Small, sunken, water-soaked lesions on a green fruit, early stages of infection, Madison, Wis., September 19, 1916; *B*, advanced stage of leaf attack, Madison, Wis., August 19, 1916.



ANTHRACNOSE ON MUSKMELONS.

A, Lesions on winter pineapple muskmelon, Madison, Wis., September 12, 1916; *B*, lesions on Orange Christiana muskmelon, Madison, Wis., September 17, 1916.

ANTHRACNOSE LESIONS ON A WATERMELON LEAF.

Madison, Wis., September 12, 1916.

ANTHRACNOSE ON CUCURBIT FRUITS.

A, *Trichosanthes colubrina*, Madison, Wis., September 6, 1916; *B*, mango melon, Madison, Wis., September 12, 1916; *C*, *Citrullus vulgaris*, Princeton, Wis., September 15, 1916.

ANTHRACNOSE LESIONS ON A FRUIT AND LEAF OF GOURD (LAGENARIA VULGARIS).

On muskmelon the leaf lesions are quite similar to those on cucumber. The attack on petiole, stem, and fruit is more severe. Petiole and stem lesions are sunken and dark colored, penetrating deeply and finally girdling. A red gummy exudate is often present. Fruit lesions vary with the host variety (Pl. III, *A* and *B*) and are less conspicuous on the netted varieties. In general, fruit lesions are oval or circular and sharply depressed (Pl. III, *B*). On some varieties the depressed epidermis may form a white background for the pink spore cushions. The latter are represented by black dots after sporulation ceases and may be arranged in concentric lines if the lesion is on the upper exposed surface of the fruit. Rotting of the fruit in the field may result from anthracnose lesions on its lower surface.

In the field, anthracnose of muskmelons is characterized by rather complete defoliation, due to the severity of petiole attack, and by the death of whole runners. Fruit lesions are also very conspicuous.

The symptoms on watermelon have been well described by Sheldon (46). On watermelon leaves the lesions are black rather than brown (Pl. IV). Petiole and stem lesions resemble those on muskmelon in appearance and severity, and as a rule such lesions cause the death of the distal portions. Infection of young fruits results in their abortion or malformation. Small lesions on such fruits are black depressed spots, soon bearing pink acervuli (Pl. V, *C*). On older fruits the lesions first appear as elevated pimples with a yellow translucent center. More common on mature fruits is the nailhead type, consisting of a rather flat-topped, circular, water-soaked elevation. Such lesions soon become sunken and bear the familiar pink spore masses on a black or cream-colored background. Lesions on the under side of the melon where it is in contact with the soil may become black and corky. It is from lesions thus situated that rotting in the field may result.

In the field, watermelon anthracnose is characterized by the scorched appearance of the foliage and by the bare, leafless areas at the centers of the hills, the stem lesions, and the disfiguration of the fruit.

On *Lagenaria vulgaris*, or gourd, the leaf lesions are also black and the petiole and stem lesions resemble those on muskmelon and watermelon. Lesions on young fruits are black, while on older white fruits the lesions are brown at first, with conspicuous yellow and water-soaked borders, and crack transversely while rather small (Pl. VI).

The symptoms on *Cucumis melo* var. *dudaim*, *C. melo* var. *flexuosus*, and the mango melon are much like those on muskmelon. On the leaves of *Cucumis dipsaceus* the spots are very light tan colored. Fruit lesions on *Trichosanthes colubrina* are illustrated in Plate V, *A*, and on the mango melon in Plate V, *B*.

THE CAUSAL ORGANISM.

TAXONOMY.

The fungus causing anthracnose as it occurred on Lagenaria fruits at Padua, Italy, was described by Passerini (16) in 1868 under the name of *Fusarium lagenarium* Pass.

Berkeley (3) in 1871 reported *Gloeosporium laeticolor* on a ripe cucumber and in 1876 (4) reported Gloeosporia on various hosts, including cucumbers and melons. He suggested that all were transferable from one host to another and that all these fungi might be identical with a fungus on gourd fruits "known to mycologists as *Gloeosporium orbiculare* Berk." Owing to imperfect description the identity of this fungus is a matter of question. In his 1910 index, Saccardo (42, v. 19) still retains this species.

In 1880 Roumeguère (41) described this disease as it occurred on muskmelons at Chalons, France. He recognized the fungus as a Gloeosporium, but at first believed it identical with a *Fusarium reticulatum* Mont. observed on watermelons in 1843, and hence named it *Gloeosporium* (*Fusarium*) *reticulatum* (Mont.) Roum. Later in the year 1880, upon the advice of Saccardo, Roumeguère (40) recognized that Passerini was the first to describe the fungus under consideration and changed the name to *Gloeosporium lagenarium* (Pass.) Sacc. and Roum. He takes no notice of Berkeley's work.

In 1882 Berkeley and Broome (5) reported a *Gloeosporium cucurbitarum* B. and Br. on Cucurbita fruits in Australia. This, Saccardo suggests, may be identical with *Gloeosporium lagenarium*, and he does not list it in his 1910 index.

In America in 1885 Ellis and Everhart (15, p. 118) list as *Gloeosporium lagenarium* Pass. a fungus found on gourds, and later *Gloeosporium lindemuthianum* S. and M. on watermelon rinds. Still later, specimens of *Gloeosporium lagenarium* (Pass.) var. *foliicolum* E. and E. upon cucumber, watermelon, and muskmelon leaves were distributed (23).

Cavara (8, p. 179) at Pavia, Italy, where Passerini first found this fungus, described in 1889 a *Colletotrichum oligochaetum* Cav., parasitic upon Lagenaria plants. He noted that this fungus differed from the earlier descriptions in that setæ were present in the acervuli and the spores were slightly smaller. Saccardo (42, v. 10, p. 469) in 1892 gave this form specific rank, but as a result of Halsted's critical examination of exsiccati it now seems certain, however, that Cavara had the same fungus previously described by Passerini and Roumeguère.

Basing his assumption upon morphological similarity and very meager cross inoculations, Halsted (23) in 1893 concluded that the fungi of bean and watermelon anthracnoses were identical. He

noted that the watermelon fungus possessed setæ and decided that it was a Colletotrichum rather than a Gloeosporium. Since, however, the specific name of the watermelon anthracnose antedated that of the bean anthracnose by seven years or more, he followed a suggestion made by Ellis in 1890 and applied the name *Colletotrichum lagenarium* (Pass.) Ell. and Hals. to the causal organisms of both melon and bean anthracnoses. While all later work indicates that Halsted was in error in assuming the identity of the anthracnose fungi of bean and melon, it appears that his change of generic name was well warranted. Saccardo, however, does not recognize this name.

Following Saccardo's opinion regarding *Colletotrichum oligochaetum*, Prillieux and Delacroix (39), publishing in 1894 on the disease as it occurred on melons, considered the above and *Gloeosporium lagenarium* as separate and distinct species and used the former name because their specimens possessed setose acervuli. Their description of the disease leaves no doubt as to its being identical with the one under consideration.

In his index of 1910, Saccardo (42, v. 19) lists both of the above species and thus recognizes two distinct fungi causing anthracnose of cucurbitaceous hosts in addition to Berkeley's *Gloeosporium orbiculare*.

In a comparative study of anthracnose fungi in 1898, Miss Stoneman (50, p. 88) cultured forms from watermelon and cucumber and concluded them to be identical. By similar tests, she found bean anthracnose to be quite distinct. She went further, however, and described (50, p. 94) as a new species *Volutella citrulli* Stoneman, a form found on a citron fruit in the Ithaca market. According to her description, this fungus differed from *Colletotrichum lagenarium* in that dense raised stromata were formed on lesions and in certain cultural features. This is recognized as a species by Saccardo (42, v. 19) in his 1910 index. Since *Colletotrichum lagenarium* is known to occur on citrons, it seems quite possible that the above may be identical with it. On the other hand, no inoculations were reported and this Volutella may have been a saprophyte.

Evidence that Halsted was mistaken in assuming the identity of bean and cucurbit anthracnoses was secured by C. O. Smith (9, p. 28) and Sheldon (46) in 1904, by Edgerton (13) in 1909, and by Krüger (28, pp. 246, 294) and Shear and Wood (45) in 1913 as a result of unsuccessful cross inoculations.

In 1910 Potebnia (38, p. 82), in Russia, published his opinion that *Colletotrichum oligochaetum* Cav. and *Gloeosporium lagenarium* (Pass.) Sacc. and Roum. were the same species, the latter being the nonsetose form.

The presence or absence of setæ in the acervuli has played a major rôle in the generic nomenclature of this fungus, since the presence of setæ forms the basis for the distinction between Colletotrichum and Gloeosporium. Krüger, in the publication above quoted (28, p. 299), faces this question as a general theme for the two genera of fungi. Miss Stoneman (50) had previously noted the sudden appearance of setæ in cultures of *Gloeosporium fructigenum*, and Krüger had also observed this phenomenon in his cultures of the above and other Gloeosporia.

Frank (19) had noted occasional lack of setæ in *Colletotrichum lindemuthianum* and Miss Stoneman had noted the same in cultures of three species, including *Colletotrichum lagenarium*. Potebnia (38) later noted the occasional lack of setæ in some acervuli of the latter fungus. Krüger (28, p. 299), working with the fungus of bean anthracnose, confirmed Frank's observation and claimed to have developed at will setose and nonsetose strains. He found that the age of the culture and the nature and moisture content of the substratum acted as controlling factors and concluded that the presence or absence of setæ can not be depended upon as a decisive generic distinction in this group of fungi. Krüger urges the necessity of some other basis of differentiation, such as cultural characters.

Shear and Wood (45) in 1913 divided the anthracnoses of 45 hosts into five species, of which three are Glomerellae and two are Gloeosporia. Of the latter, one is *G. lagenarium* (Pass.) Sacc. and Roum. on watermelon, cucumber, and squash. In the whole group of fungi studied they noticed (45, p. 64) that there was great variation as to the presence, absence, and abundance of setæ in cultures from the same host and even from the same spore. They also noticed that there was variation in size, length, and septation of setæ. In the form from cucumber they noted setæ sometimes present in culture, and in the form from watermelon setæ were abundant in lesions and in culture. Setæ were sometimes present in squash lesions.

Carsner[1] in 1914, using a strain isolated from a muskmelon fruit, found no setæ present and was inclined to retain the name Gloeosporium.

Eriksson (16, p. 125) studied the fungus causing the disease on greenhouse cucumbers in Sweden, found setæ present, and as a result of a rather careful review of the literature retained Halsted's name, Colletotrichum.

To sum up the situation relative to the nomenclature of this fungus, we have difficulties arising from several sources: (1) Synonyms, of which *Colletotrichum oligochaetum* Cav. is an example. (2) Fungi of uncertain identity, such as *Gloeosporium orbiculare* Berk. and *Volutella citrulli* Stoneman. (3) Halsted's theory that the bean and cucur-

[1] Carsner, E. Op. cit.

bit anthracnoses are caused by the same fungus. (4) The difficulty in distinguishing the genera Colletotrichum and Gloeosporium on the basis of the presence or absence of setæ. (5) The morphological similarity of conidial stages of all anthracnose fungi and the necessity of knowing the perfect stages in order to make specific distinctions. (6) The question as to the validity of species erected upon the basis of host relationships.

The evidence that the bean and cucumber anthracnoses are not identical has been augmented by numerous observations and cross inoculations made in the course of the present work, and there seems to be no further question as to the specific distinctness of the two. Several unsuccessful attempts have been made to cross inoculate with the causal organisms, and exposure of beans to natural infection has yielded only negative results.

The fungus of cucurbit anthracnose shows quite constant and distinctive cultural characters. The host relationships seem quite definite and check up with cultural and morphological characters. Upon the basis of host relationships as laid down by Edgerton (13), there seems to be no reason to doubt that there is one well-defined species of fungus causing the disease on the hosts listed. Since setæ are present in all of the strains studied during the course of this work, it seems quite logical to retain until a perfect stage is discovered Halsted's name *Colletotrichum lagenarium*, with the understanding that it holds for only one of the two fungi to which Halsted applied it. If, however, not much importance is attached to the presence or absence of setæ, the earlier name *Gloeosporium lagenarium* (Pass.) Sacc. and Roum. may be retained. It appears to be purely a matter of arbitrary choice between the two names.

SYNONOMY.

Colletotrichum lagenarium (Pass.) Ell. and Hals., 1893, in Bul. Torrey Bot. Club, v. 20, p. 246–250; or

Gloeosporium lagenarium (Pass.) Sacc. and Roum., 1880, in Rev. Mycol., année 2, p. 200–202.

Fusarium lagenarium Passerini, 1868, in Erbario Crittigamico Italiano, s. 2, no. 148.

Gloeosporium reticulatum Roumeguère, 1880, in Rev. Mycol., année 2, p.169–172.

Colletotrichum oligochaetum Cavara, 1889, in Rev. Mycol., année 11, p. 191.

RELATIONSHIP UNCERTAIN.

Gloeosporium orbiculare Berkeley, 1876.

Gloeosporium cucurbitarum Berkeley and Broome, 1882.

Volutella citrulli Stoneman, 1898.

MORPHOLOGY AND CULTURAL CHARACTERS.

MORPHOLOGY.

The morphology of this fungus has been described rather completely by various workers, such as Roumeguère (41), Cavara (8),

Halsted (23), Prillieux and Delacroix (39), Stoneman (50), Sheldon (46), Potebnia (38), Shear and Wood (45), and Eriksson (16). Potebnia gives an especially good description.

Mycelium.—The mycelial characters vary greatly with age and substratum. At first the mycelium is colorless, thin walled, septate, and quite uniformly cylindrical. Many of the cells later increase in diameter about threefold and tend to become thick walled and dark brown in color, resembling intercalary chlamydospores. Oil drops are commonly present in old mycelium. In culture, the mycelium is first colorless, then pink, and finally black. In host tissue the pink coloration is sometimes seen, and the blackening is quite commonly produced in fruit lesions. The brown, thick-walled, large-celled mycelium occurs commonly in host tissue.

Acervuli.—The mycelial filaments tend to aggregate at certain points, branch, intertwine, and send out a palisade layer of short colorless conidiophores. The extent of stromatal development previous to sporulation varies greatly and is apparently greater in culture than in host tissue. The color of this stromatic tissue is brown or black.

Setæ.—Scattered about among the conidiophores are the long 2–3 septate, brown, thick-walled bristles, or setæ, varying in length from 90 to 120 μ and tapering toward a blunt point. The setæ may be much longer under certain conditions. The number in each acervulus varies greatly and is given as high as 24 to 36 by Potebnia.

Spores.—From the tips of the conidiophores the spores are budded off apically, one at a time, and pile up in a pink slimy heap on top of the acervulus. The spores are embedded in a sticky water-soluble matrix, and the heaps are often as high as the setæ, the latter apparently serving as supports to hold the spore mass in place. The spores are one celled, hyaline, oblong or ovate-oblong, and slightly pointed at one end. Spores vary considerably in shape. Their size is about 13 to 19 μ by 4 to 6 μ. Usually two or three vacuoles are present. The spores are pink in mass.

Sclerotial bodies.—These are usually the result of the further development of the stromata or bases of the acervuli, in which the whole mass becomes considerably enlarged and black in color. The size of these sclerotial masses varies greatly, as does also the degree of their development. Sclerotial masses are formed abundantly in culture and in fruit lesions. Sheldon (46) describes these in detail. In culture the spore mass may dry down and remain as part of the protruding sclerotium. In fruit lesions the spores are washed away and only the black stroma remains, forming the black spots in the fruit lesions previously described.

Appressoria.—Normally, a germinating spore on a firm or hard substratum forms an appressorium at the tip of each germ tube or

branch thereof. The appressoria are brown, thick-walled, ovoid to spherical cells, in general appearance not unlike the intercalary chlamydospores of the mycelium. The analogy between these appressoria and chlamydospores is strikingly shown in cases where appressoria are formed in series, one apparently budding out to form another. The appressorium may taper slightly toward the point of attachment of the germ tube and may be flattened on the side in contact with the substratum.

A definite round germ pore is found in the center of the lower side of an appressorium from which leaf penetration has occurred. For all of the strains studied, the size and shape of the appressoria seem to be quite uniform. In a few cases appressoria have been observed to increase considerably in size and become two celled.

CULTURAL CHARACTERS.

Carsner [1] has described the cultural characters somewhat at length. No effort has been made in this work to compare the growth on different media. For general purposes a $2\frac{1}{2}$ per cent water agar containing 2 per cent of dextrose has proved very useful. Besides this, potato, bean, and apple-twig agar have been used. The fungus grows rapidly and is easily cultured.

Isolation from diseased specimens was usually accomplished by first transferring spores from the acervuli to a drop of sterile water on a flamed slide. From this drop loop inoculations were made into tubes of melted agar. Plates were poured and transfers made from single colonies developing therein. Many of the strains were grown from a single spore.

There is great variation among different strains in the amount of aerial growth, the extent of the blackening, and the abundance of sporulation. Cultural characteristics tend to change during prolonged propagation, as has been noted in other anthracnose fungi by Edgerton (12, p. 393). The strains longest in culture seem to sporulate most abundantly. Sporulation can be readily secured by the use of sterilized segments of cucumber stems.

In test-tube culture the mycelium is first white or colorless, later pink, and finally black. The aerial growth usually becomes prostrate quite promptly. Acervuli appear first as black points and sporulation occurs within a week. The pink spore masses may be formed for several weeks. In old cultures the black sclerotial bodies are prominent on the surface and also scattered through the medium to some depth. General blackening of the mycelium may extend to some depth also.

[1] Carsner, E. Op. cit.

PHYSIOLOGY.

Only preliminary work was done on the physiological phases of the problem, and since, as a rule, the tests were not made in duplicate, only a brief summary of the results will be presented.

NUTRITION.[1]

In studying the nutrition of the fungus, use was made of a standard nutrient solution containing molecular ÷ 8 ammonium nitrate, molecular ÷ 20 potassium acid phosphate, molecular ÷ 50 magnesium sulphate, molecular ÷ 1,000,000 iron chlorid, and about 5 per cent sucrose. By substitutions within this formula, the effect of the omission of an element was determined. The fungus was grown in parallel series with *Aspergillus niger* in flasks containing about 50 c. c. of the medium. Dry-weight yields were used as criteria.

Using the full nutrient solution, it was found that in 24 days Colletotrichum produced only about one-twentieth as much dry-weight yield as did Aspergillus in 15 days, but after a much longer interval the yield of Colletotrichum was almost as great. No sporulation of the latter was secured and it was evident that a liquid medium was not well adapted to the development of Colletotrichum.

As a result of substitutions in the formula it was found that carbon, nitrogen, potassium, and phosphorus were essential to the growth of Colletotrichum, while magnesium was needed in only very minute quantities. Sulphur and iron were not needed in excess of the amounts present as possible impurities. Colletotrichum seems to be less sensitive to a lack of magnesium and sulphur than Aspergillus and more sensitive to a deficiency of potassium and phosphorus.

Substituting other carbohydrates for sucrose, so that an equal amount of carbon was present, it was found that Colletotrichum, unlike Aspergillus, uses cornstarch to exceptional advantage. The other carbon sources, listed in descending order of suitability, are inulin, maltose, dextrose and galactose, salicin, glycerol, and lactose. Sucrose should probably be ranked with the dextrose and galactose. For Aspergillus the order would be sucrose, inulin and maltose, dextrose, cornstarch, galactose, tannin, glycerol, lactose, and salicin. Colletotrichum was unable to utilize tannin.

Further proof of the preference of Colletotrichum for the more complex carbon compounds was afforded by its abundant development on xylan as a carbon source. Using xylan prepared from straw in place of sucrose in an amount such that only one-fourth as much carbon was available as in the full nutrient solution, a similar dry-weight yield of fungus mycelium was obtained. Xylan appears to be fully as suitable a carbon source as cornstarch.

[1] The tests relative to nutrition and the effect of copper sulphate were made under the direction of Prof. J. B. Overton and Dr. J. P. Bennett, of the University of Wisconsin.

Cellulose may also be utilized as a sole source of carbon. Pure cellulose jelly prepared from absorbent cotton and substituted for sucrose supported a growth of mycelium which yielded in several months a dry weight greater than that of the cellulose added. Further proof of the ability of Colletotrichum to dissolve cellulose was furnished by the clearing of the turbidity in an unslanted test tube of cellulose agar to a depth of 1 cm. beneath the colony. This suggests that a cellulose-dissolving enzym was secreted, which acted in advance of the mycelium. In a similar tube containing sucrose in addition to the cellulose, growth was more vigorous, but little or no visible disappearance of the cellulose was noted. This indicates that the presence of sucrose may have inhibited the utilization of cellulose.

EFFECT OF COPPER SULPHATE.

Using the full nutrient solution, the influence of very low concentrations of copper sulphate upon the growth of Colletotrichum and Aspergillus was studied. Colletotrichum proved to be much more sensitive to copper sulphate. Whereas Aspergillus showed marked increase of yield in a concentration of molecular ÷ 16,000, toxic effects in molecular ÷ 1,000 and ability to grow in the presence of molecular ÷ 250, Colletotrichum was only stimulated slightly, if at all, by concentrations of copper sulphate as low as molecular ÷ 128,000 and showed toxic effects in molecular ÷ 64,000 and total inhibition of growth in molecular + 2,000.

SPORE GERMINATION.

In poured plates of agar at room temperature, spores germinate within five hours. In drops of distilled water on slides at room temperature, germination usually occurs within 24 hours, although this time varies greatly. In some cases abundant germination occurred within 10 hours and appressorium formation within 20 hours. In pipette-dropper inoculations of leaves in the greenhouse, it has been found that abundant appressorium formation occurred within 24 hours.

In general, germination is favored by the presence of some nutrient material and a 2 per cent dextrose solution or a prune decoction made by steaming 10 grams of prune flesh in 1,000 c. c. of distilled water has proved very useful. An abundant oxygen supply seems quite essential. Germination is more prompt about the edge of drops containing spores and is more vigorous in exposed drops than in Van Tieghem cells.

To determine the effect of such factors as temperature, food supply, and aeration upon germination, a series of tests was run. This consisted of a quadruplicate series of hanging drops in Van Tieghem cells and exposed drops on flamed slides in prune decoction and in dis-

tilled water inoculated with spores and incubated at thirteen different temperatures between 3° and 31° C. secured in an Altmann compartment incubator and in other constant-temperature incubators. The results are presented in Table II.

TABLE II.—*Relation of temperature, oxygen supply, and food supply to spore germination and appressorium formation of Colletotrichum lagenarium.*

[Abbreviations: ab = abundant, ap = appressoria, d = day or days, g = germination, gt = germ tubes, hr = hours, mar = marginal, num = numerous, pc = per cent, spo = sporulation, sw = swelling, tg = trace of germination.]

Temperature (C.).	Distilled water.		Prune decoction.	
	Hanging drop.	Exposed drop.	Hanging drop.	Exposed drop.
2° to 3°	19 d, no g	19 d, no g	19 d, no g	19 d, some swollen.
3° to 4°		10 d, no g	10 d, no g	7 d, sw; 19 d, a few short gt.
6° to 7°	10 d, no g; 19 d, sw	5 d, no g; 7 d, sw; 10 d, a few budlike gt.	19 d, no g	1 d, no g; 2 d, tg; 5 d, num short gt; 7 d, 50 pc short budlike gt.
9° to 10°		3 d, no g; 19 d, budding gt.	19 d, no g	1 d, no g; 2 d, 3 pc g; 7 d, 90 pc g, short gt, ap ab.
11° to 12°	2 d, sw; 10 d, no g	2 d, 10 pc g; 3 d, ap ab; 5 d, 80 pc g.	2 d, sw; 10 d, sw, no gt.	10 hr, tg; 1 d, 1 pc g, short gt; 2 d, 90 pc g; 3 d, ap general.
12.5° to 14°		1 d, 1 pc short slender gt; 2 d, 10 pc g, ap present.	1 d, no g; 2 d, swollen (septate); 7 d, 95 pc same, 5 pc normal g.	10 hr, tg; 1 d, 5 pc g; 2 d, a few ap; 10 d, acervuli spo.
14°	1 d, no g; 2 d, 5 pc g; 5 d, ap formed.	1 d, tg; 2 d, 90 pc g, ap num (mar).	2 d, no g (cell found air tight); 5 d, no g.	1 d, 20 pc g, gt robust; 2 d, 90 pc g, a few ap; 5 d, ap num, acervuli forming.
15°		9 hr, 1 spore germinating; 1 d, 5 pc g, a few ap.	1 d, 1 pc g; 2 d, 5 pc g, long gt; 3 d, no ap; 7 d, acervuli; 10 d, spo.	9 hr, tg; 1 d, 5 pc g, ap present (mar); 2 d, ap. num; 7 d, spo.
15° to 17°	2 d, no g	1 d, 20 pc g, ap present; 2 d, ap num (mar).	11 hr, 2 spores germinating; 1 d, 1 pc g; 2 d, 3 pc g, ap present; 4 d, acervuli forming; 5 d, no spo.	9 hr, 1 pc g; 1 d, 40 pc g, ap present; 3 d, ap num; 7 d, spo.
18° to 20°	10 hr, 2 pc g; 1 d, 10 pc g; 3 d, no ap; 4 d, ap num.	10 hr, 1 spore germinating; 1 d, 5 to 10 pc g, ap num (mar); 2 d, 10 pc g.	1 d, 90 pc g; 2 d, no ap; 3 d, ap num.	9 hr, 3 pc g; 1 d, 10 pc g, ap present (mar); 3 d, acervuli forming; 4 d, spo.
22°	8½ hr, 5 pc g; 1 d, 20 pc g, gt slender, a few ap (mar); 2 d, ap num.	1 d, ab g, ap present; 2 d, ap num (mar).	1 d, 90 pc g; 4 d, spo, no ap.	8 hr, 10 pc g; 1 d, 20 pc g; 2 d, ap; 4 d, spo.
26½° to 27½°	10 hr, 20 pc g; 1 d, 30 pc g; 3 d, a few long-stalked ap.	8 hr, 10 pc g, 1 d, 25 pc g, ap (mar); 2 d, ap num.	10 hr, sw; 1 d, 95 pc g; 5 d, no ap; 7 d, spo.	8 hr, 10 to 20 pc g; 1 d, 75 pc g; 2 d, ap present; 4 d, spo.
30° to 31°	9½ hr, lateral budlike gt; 20 hr, 40 pc g; 3 d extremely swollen budded spores or "involution forms"; no ap.	8 hr, tg; 1 d, 5 pc g; 4 d, 50 pc g; 5 d, no ap.	9 hr, sw; 20 hr, 95 pc g; 2 d, extreme sw of spores and mycelium; 3 d, no ap.	8 hr, 10 to 20 pc g; 1 d, 95 pc g, colonies visible to unaided eye, no ap; 5 d, no ap, no darkening.
44°		No g		No g.

More vigorous germination was obtained in prune decoction than in water, and in either medium the exposed drops furnished better conditions for germination than the hanging drops. The latter is attributed to lack of aeration in the Van Tieghem cells, although, in preparing these, care was exercised not to have the vaseline seal continuous around the edge of the ring. At the rather high tempera-

ture of 30° C. swollen "involution forms" were produced in the Van Tieghem cells.

The minimum temperature permitting germination was 4° C. in exposed drops of prune decoction, 7° in exposed drops of water, and 14° in hanging drops in either medium. The optimum temperature for germination apparently lies between 22° and 27° C. Edgerton (14) found that the optimum temperature for mycelial growth was 24° C. It is of interest to note here that at temperatures of 20°, 22°, and 27° C. the fungus in the exposed drop of prune juice completed its life cycle in four days with the production of sporulating acervuli. In the hanging drop of the same medium at 27° and in exposed drops at 16° C., seven days were required to complete the cycle.

Germination is favored, therefore, by the presence of nutrient material, by a good oxygen supply or at least good aeration, and by temperatures of 22° to 27° C.

APPRESSORIUM FORMATION.

As to the factors influencing the formation of appressoria in general, De Bary (2) held that, in Sclerotinia, appressoria were the result of a contact stimulus, and his opinion was corroborated by Büsgen (7), who worked with several parasitic fungi. Halsted (24) found that appressoria were generally produced by Gloeosporium and Colletotrichum. American writers in general have called them secondary spores or chlamydospores (26). Hasselbring (26), working with the fungus of apple bitter-rot, found that appressoria were formed only as a result of a contact stimulus in a medium poor in food supply. Appressoria were never formed until the germ tube came into contact with the glass, and not even then when in a nutrient medium. He found also that the appressoria tended to adhere to the glass.

In the course of this work some effort was made to throw further light on the factors controlling appressorium formation. In the germination tests mentioned above it was noted that the appressoria were usually but not always formed in contact with the glass. They occurred in the hanging drops usually up against the glass and were not as abundant as in the exposed drops. Appressoria were formed just as commonly in the nutrient prune decoction as in the distilled water.

It was noted that the formation of appressoria in exposed drops, as well as in hanging drops, occurred most readily about the margin of the drop. Furthermore, their formation in hanging drops of water was more general than in the hanging drops of prune decoction. In exposed drops of prune juice appressoria were formed at temperatures from 9° to 27° C.; in hanging drops, only between 17° and 20°. In exposed drops of water appressoria were formed between 12° and

27° C. and in hanging drops between 14° and 27°. In no case were any formed at the high temperature of 31°. The point to be noted here is limited appressorium formation in the hanging drops of the nutrient medium.

To account for certain of these phenomena it seems quite reasonable to assume that a rather abundant oxygen supply is one of the essentials for appressorium formation. It is likely that the spores about the extreme edge of a drop would be better supplied with oxygen than those in the interior. Exposed drops have an abundant oxygen supply. In Van Tieghem cells, where the oxygen supply is limited, it is quite likely that the demand for oxygen would be greater and hence the supply more quickly used up in the nutrient medium than in pure water, since more rapid growth occurs in the former.

With this fungus, then, the presence of food material does not seem to inhibit appressorium formation. The contact stimulus is apparently necessary as a rule, and a liberal oxygen supply seems to be favorable to appressorium formation. The function of appressoria will be considered later in connection with the relation of the fungus to the host tissue.

PATHOGENICITY.

Whether or not we accept Edgerton's (13) basis of species distinction by host range, it is essential from the phytopathological standpoint to know the exact host range of this fungus.

Under general field conditions the disease has been observed commonly on cucumbers, watermelons, muskmelons, and Lagenaria gourds. Experimental evidence of the identity of the fungus in these cases is furnished by the cross inoculations made by Sheldon (46), who secured infection of cucumber, watermelon, muskmelon, and gourd plants with a strain isolated from watermelon, and by Carsner,[1] who secured infection of cucumber plants with a strain from muskmelon.

During the course of this work, successful cross inoculations have been made from watermelon to cucumber, from cucumber to watermelon and muskmelon, and from muskmelon to cucumber. Leaf infection of vigorous plants was secured in all cases. Using the fungus from cucumber, successful cross inoculations have been made in the field upon eight varieties of muskmelon, upon *Cucumis anguria*, *C. melo* var. *flexuosus*, *C. melo* var. *dudaim* and *C. dipsaceus*, and upon *Trichosanthes colubrina* and *Benincasa cerifera*. In these inoculations a spore suspension was applied with an atomizer. Out of three series made upon about 40 varieties on August 4, 10, and 13, 1916, success was obtained only in the inoculations of August 13.

Out of a large variety of cucurbits exposed to natural infection by drainage water under conditions which practically precluded the pos-

[1] Carsner, E. Op. cit.

sibility of confusion with infection from any source other than badly diseased cucumbers, the list of hosts is increased by the addition of several varieties of cucumber, two varieties of muskmelon, citron, seven Lagenaria gourds, and *Cucumis anguria* var. *grossulariae*. Exposure to natural infection in an experimental field at Plymouth, Ind., in 1916 adds to this list of hosts *Momordica balsamina* and *M. charantia*.

Repeated attempts to inoculate 6 varieties of squash, 2 varieties of pumpkin, and 12 varieties of Cucurbita gourds yielded only negative results, as did also attempts to inoculate *Echinocystis lobata*, *Bryonopsis laciniosa*, 3 Momordica species, and a species of Luffa.

To determine just how the fungus might react toward squash, drops of a spore suspension were placed on the leaves of a squash plant in the greenhouse. Except for a slightly yellowed area on one old leaf no lesions developed. Microscopic examination of material from this yellowed area, fixed according to methods to be described later, showed that the fungus had penetrated the epidermal cells but had gone no farther.

Exposure to the same epidemic of natural infection above noted gave negative results in the following: Early Russian cucumber, Giant Pera cucumber, pumpkins (2 varieties), squash (6 varieties), *Bryonopsis laciniosa*, *Luffa* sp., and 16 varieties of Cucurbita gourds.

Using cultures from watermelon, Sheldon (46) was unable to infect squash, pumpkin, and wax-bean plants. Edgerton (12) and Taubenhaus (51) were unable to infect apple fruits. The latter also secured no infection of sweet-pea plants. In the present investigation no success was obtained in an attempt to infect apple fruits. Inoculations on five varieties of beans in the field gave negative results, as did also exposure of these varieties to natural infection during an entire season.

Following is a list of susceptible host varieties as noted during the course of this investigation:

Cucumbers.—Heinz Chicago Pickling, Nichols' Medium Green, Thorburn's Klondike White Spine, Japanese Climbing, Lemon, Boston, Jersey, Arlington, Milwaukee, Livingston, Emerald, Davis Perfect, Improved Long Green, Early Fortune, Telegraph, Vaughan's Prolific, Carter Model.

Muskmelons.—Banquet, Long Yellow, Shumway Giant, Orange Christiana, Hybrid Casaba, Hackensack, Rocky Ford, Winter Pineapple, Banana, Mango.

Watermelons.—Watson, Pierson, Red-Seed Citron.

Lagenaria gourds.—Bottle, Dishcloth, Knob Kerrie, Dipper, Hercules Club, Sugar Trough, Calabash.

Other cucurbits.—*Cucumis anguria*, *C. anguria* var. *grossulariae* (fruit only), *C. dipsaceus*, *C. melo* var. *flexuosus*, *C. melo* var. *dudaim*, *Trichosanthes colubrina*, *Benincasa cerifera*. Reported on *Momordica balsamina* and *M. charantia*.

RELATION TO OTHER ANTHRACNOSE FUNGI.

Upon the basis of Edgerton's theory of species determination by host range, it is of interest to note the reaction of anthracnose fungi from other than cucurbitaceous hosts to the latter. Using the fungus from bean Frank (19) failed to infect a cucumber fruit and C. O. Smith (9) secured no infection of plants in the cases of cucumber, pumpkin, squash, muskmelon, and watermelon. Likewise, Edgerton (13) was unable to obtain infection with the bean fungus upon cucumber plants and fruit, and Krüger (28, p. 246, 294) was unable to infect cucumbers with the fungus from bean. Shear and Wood (45) were also unsuccessful in their inoculations of watermelon, squash, and pumpkin fruits with the bean fungus, but were successful in their inoculations of the fruits of watermelon, squash, and pumpkin with the anthracnose fungus from grapes and also in their inoculations of watermelon and pumpkin fruits with the fungus from guava. Halsted (23) secured infection of a citron fruit with the fungus from bean.

It will be noted that in no case did the anthracnose fungus from another host infect cucurbitaceous plants. In cases of fruit infection it must be borne in mind that the fruit to some extent resembles a nonliving substratum and that successful fruit infection is not proof of active parasitism.

In the course of the present work, atomizer inoculations of cucumber plants in the field with spore suspensions from cultures of bean, cotton, banana, and fig anthracnoses yielded only negative results.

There seems to be no positive evidence that fungi causing anthracnose of hosts other than cucurbits are physiologically identical with the form under consideration, and, as was noted in the consideration of the pathogenicity of *Colletotrichum lagenarium*, this fungus causes anthracnose of cucurbits only.

RELATION OF THE FUNGUS TO THE HOST TISSUE.

Concerning the relation of the fungus to the host tissue, attention has been chiefly focused upon the mode of penetration of the host epidermis and the effect of the advancing mycelium upon the host cells.

PENETRATION.

To determine the method of host penetration, drops of spore suspensions were placed in ink circles on leaves of cucumber plants in the greenhouse, and at intervals thereafter these inoculated leaf areas were cut out with scissors, fixed in 10 per cent HCl, and cleared by storing in a saturated solution of chloral hydrate. This was followed by clearing in 3 per cent KOH, and in some cases staining in Delafield's hæmatoxylin. Glycerin mounts were made and the surface of the epidermis was carefully examined under the microscope.

As a result of the examination of a considerable number of these preparations, it was found that within 24 hours after inoculation appressoria had been formed in abundance, usually one to each spore, sessile, or on short germ tubes. These were particularly numerous

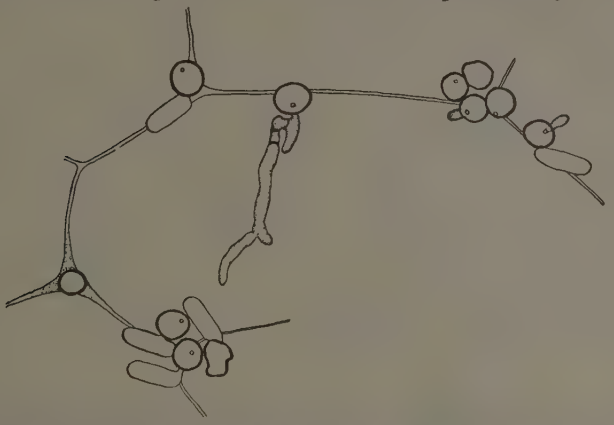


FIG. 1.—Surface view of a portion of a cucumber-leaf epidermal cell, showing appressoria and penetration tubes, six days after inoculation. In five instances the empty spore case is shown lying near the appressorium. The circular germ pores can be seen in the appressoria. Two appressoria (upper right) have formed short penetration tubes. Another has produced considerable mycelial growth within the host cell. (Camera-lucida drawing; magnified about 395 times.)

in the vein depressions and in the depressions bounding the epidermal cells. No signs of host penetration were found in this material or in material fixed 44 hours after inoculation.

In leaf areas fixed 65 hours after inoculation, penetration tubes were commonly visible within the epidermal cells underneath the appressoria and a small round pore was visible in the wall of the appressorium next to the host cuticle (fig. 1). Hasselbring noted similar pores in the appressoria of the apple bitter-rot fungus. In material fixed 121 hours after inoculation, when macroscopic symptoms were already apparent, the penetration tubes were found under a large percentage of the appressoria, and in many cases considerable mycelium was found in the epidermal cells underneath (fig. 2).

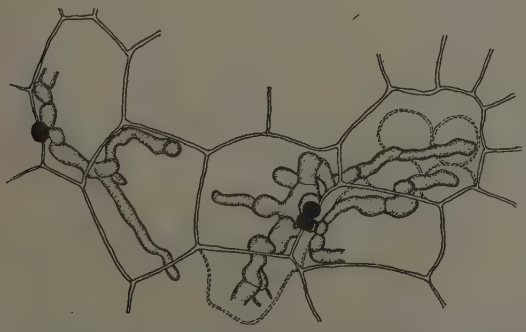


FIG. 2.—Surface view of epidermal cells of a cucumber leaf, showing intracellular mycelium from three appressoria, six days after inoculation. (Camera-lucida drawing.)

Incipient and advanced stages of penetration, as seen in surface view, are illustrated in figure 1. The appressoria are seen to be quite uniform in size, shape, and size and shape of germ pore. Although this material was subjected to several hours' washing in running water, the appressoria remained adherent to the cuticle. From a study of these preparations it appears that penetration never occurs except from an appressorium and that penetration occurs directly through the cuticle. From this, it seems safe to conclude that the appressoria have a definite function in connection with host penetration. No cases of stomatal entrance were found. A case was found in which a spore lying directly over a stomatal opening formed an appressorium at one side.

To better understand the mode of penetration, material similar to that above described was fixed in 10 per cent HCl, acetic alcohol, or Gilson's fixative, embedded, sectioned, and stained in the triple stain or Haidenhain's iron alum hæmatoxylin. Numerous penetrations were found in sections from fixations made 65 and 121 hours

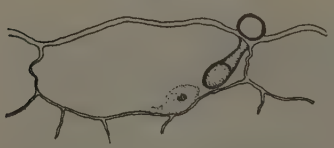


FIG. 3.—Cross section of leaf epidermis, showing penetration of epidermal cell from an appressorium, 65 hours after inoculation. The tip of the penetration tube is swollen. (Camera-lucida drawing; magnified about 575 times.)

FIG. 4.—Cross section of the outer wall of a leaf epidermal cell, showing an incipient stage of penetration from an appressorium, 65 hours after inoculation. (Camera-lucida drawing; magnified about 1,300 times.)

after inoculation, and types are illustrated in figures 3 and 4. The latter represents an incipient stage. Here, again, it is seen that direct penetration of the cuticle and outer wall takes place. Stomatal entry does not occur. In one case (fig. 5) an appressorium has been formed in the stomatal pore and penetration of a guard cell has followed, rather than a direct invasion of the substomatal chamber. In figure 6, showing an apparent stomatal penetration, close examination reveals that the lower guard cell was pierced by the penetration tube, which then emerged into the substomatal chamber.

Appressoria may be distinctly flattened on the side next to the cuticle (fig. 6), and all are in very close contact with the latter. The penetration tube varies in shape, but is usually more or less club shaped, owing to the fact that it becomes swollen after gaining access to the lumen of the cell (fig. 3). In figure 7 it is quite evident that the contents of the appressorium have entered the penetration tube. In no case has it been possible to trace the course of the penetration tube through the cuticle and underlying wall layers.

In some cases of delayed or, perhaps, inhibited penetration, there is a swelling and change of staining reaction of the cell wall under the appressorium, characterized by retention of the safranin in the triple stain (fig. 8).

The mode of fruit penetration appears to be somewhat similar. Sections from fixations made two weeks after inoculation show numerous examples of direct penetration of the cuticle and thick outer wall, but no cases of subsequent mycelial development. In view of this fact, it seems likely that these are abortive or inhibited penetrations. Fruit penetration is illustrated in figure 9. Here the penetration tube is not as clearly distinguishable as in the leaf sections, and only the swollen tip is to be seen. There is indication of a splitting apart of the wall layers in some cases, and all cases are characterized by a marked retention of the safranin in the inner wall layer under the appressorium (fig. 9).

FIG. 5.—Cross section through a stoma, showing an appressorium formed directly within the stomatal pore. Penetration of a guard cell has occurred, 65 hours after inoculation. (Camera-lucida drawing; magnified about 1,300 times.)

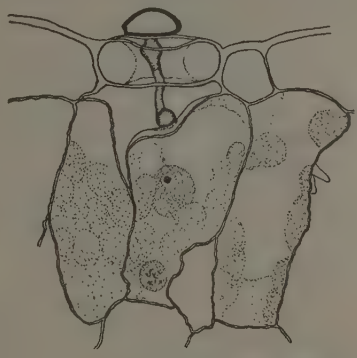


FIG. 6.—Cross section of leaf epidermis and palisade tissue, showing penetration of a stomatal guard cell from an appressorium, emergence of penetration tube into substomatal chamber, and indentation of palisade cell by the swollen tip of the tube. The wall of the palisade cell is swollen and retains safranin in the area in contact with the fungus; 65 hours after inoculation. (Camera-lucida drawing; magnified about 570 times.)

FIG. 7.—Cross section of leaf epidermis, showing penetration of an epidermal cell, 121 hours after inoculation. The contents of the appressorium have apparently passed into the penetration tube. Mycelial growth has begun from the swollen tip of the latter. Division of the host nucleus has occurred as a result of the fungous invasion. (Camera-lucida drawing; magnified about 615 times.)

The exact method of cuticle penetration is not yet understood. It is not known whether the penetration of the cuticle proper is due to mechanical pressure alone, as Blackman and Welsford (6) find in Botrytis, or whether there is some softening in advance of actual

entry, or, at least, in conjunction with it, as De Bary (2), Büsgen (7), and Miyoshi (30) have concluded for certain parasitic fungi. There is no evidence of a cavity in the cell wall, indicating distinct solvent action, such as Hasselbring found in the barberry (26). Nor was any indentation and rupture of the cuticle discovered, such as Blackman and Welsford describe. However, the germ pore is very small, and swelling of the contents of the appressorium could exert considerable pressure on the cuticle at that point lying directly beneath the germ pore. This is suggested in figure 10, a case in which the appressorium has apparently forced itself loose. In figure 6 the palisade cell wall is clearly seen to be indented by the pressure of the hyphal tip. On the other hand, the swelling of the wall under an apparently unbroken cuticle has been noted (fig. 8), and swelling of an area of the palisade cell wall about the point in contact with the fungus is also evident in figure 6.

FIG. 8.—Cross section of the outer wall of a leaf epidermal cell, showing swelling under an appressorium accompanied by retention of safranin stain, 65 hours after inoculation. No penetration tube is visible. (Camera-lucida drawing; magnified about 1,300 times.)

FIG. 9.—Cross section of the upper end of a cucumber-fruit epidermal cell, showing penetration from an appressorium, two weeks after inoculation. Retention of the safranin stain is shown in the inner wall layer under the appressorium. (Camera-lucida drawing; magnified about 540 times.)

FIG. 10.—Cross section of the outer wall of a leaf epidermal cell, showing an appressorium apparently forced off by pressure exerted by the penetration tube, 121 hours after inoculation. (Camera-lucida drawing; magnified about 1,300 times.)

To summarize, penetration takes place directly through the cuticle from appressoria in close contact with the latter and provided with a small round germ pore. The exact method of cuticle penetration has not been determined.

EFFECT ON INVADED CELLS.

In the leaf sections from material fixed 121 hours after inoculation incipient lesions are found to be characterized by marked shrinkage and collapse of the epidermal and palisade cells. These cells stain deeply with the hæmatoxylin. The mycelium is almost entirely intracellular (fig. 11). In some cases the appressorium from which infection occurred is still to be seen (fig. 11). In sections of a stem lesion collapse of the collenchyma cells is plainly visible. In razor sections of diseased stems and

petioles the intracellular mycelium about the advancing edges of the lesion is easily seen without staining.

As to the effect on host cells besides the shrinkage and the staining reaction above noted, there appears to be an enlargement of the host nucleus, often followed by its division (figs. 6 and 7) and in some cases by cell division (fig. 11). In cucumber-stem lesions there seems to be a tendency toward callus formation.

To sum up, it may be said that the mycelium is intracellular and that shrinkage and collapse of the invaded host cells occur. There are indications of a stimulus to cell division.

LIFE HISTORY OF THE CAUSAL ORGANISM IN RELATION TO THE DISEASE.

SEASONAL DEVELOPMENT.

Previous observations indicate that anthracnose is a disease which becomes serious only rather late in the growing season of its host. Observations made during the course of this work lead to the same conclusion. This appears to be due to the mode of origin of the disease in rather isolated and restricted centers in each field, from which subsequent spread, dependent upon heavy rains, is rather slow until a considerable reservoir of infection has developed. The strict relation of epidemics of this disease to wet weather is well recognized in the literature (41, 43, 44), and the importance of climatic conditions can not be overestimated.

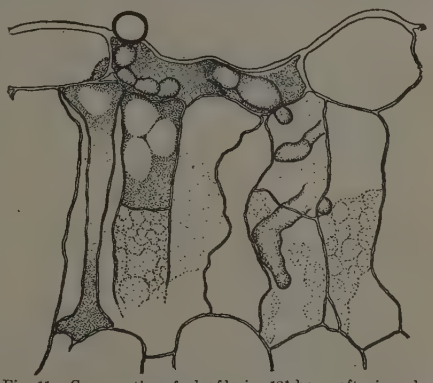


Fig. 11.—Cross section of a leaf lesion 121 hours after inoculation. Appressorium shown. Mycelium intracellular. The division of invaded palisade cells and collapse of epidermal and palisade cells are visible. (Camera-lucida drawing; magnified about 570 times.)

DEVELOPMENT IN 1915.

Observations upon the disease as it occurs among cucumbers were made during the summers of 1915, 1916, and 1917. The summer of 1915 was cool with frequent and abundant rainfall. At Princeton, Wis., the disease was first noted in a 2-acre experimental field on July 14. This infection was confined to lesions on the first and second leaves of two adjacent plants planted seven weeks previously. The next day the disease was found in a neighboring private field. By July 20 several centers of anthracnose were noted in the latter field. On July 21 anthracnose was found in another private field in town.

On July 26 a second center was found in the experimental field with secondary infection present. The rains of July 17, 19, and 21 probably account for this secondary infection, as well as that noted about the centers in the neighboring private field. An inspection of all of the 217 plats in the experimental field on July 28 resulted in the discovery of the disease in only two other plats. One week later scattered anthracnose lesions were noted in five more plats. It is of interest to note that up to this time all the anthracnose infection in this field was confined to one area consisting of 15 adjacent plats.

A careful inspection of all the plats on August 9 and 10 revealed anthracnose in five more plats in the area mentioned above and tertiary infection was noted about the old centers. Due probably to the rains of August 1 to 5, there followed a very rapid spread of the disease. An inspection of the field on August 17 and 18 revealed anthracnose in 45 plats outside of the diseased areas already alluded to. In two of these cases the infection was in apparent original centers. Thus, the most rapid spread in 1915 seems to have occurred prior to the middle of August, and it will be remembered that wet weather prevailed the first five days of that month. A frost killed the vines on August 28.

DEVELOPMENT IN 1916.

In the summer of 1916 the cucumber experimental work was located at Madison. With five rather scattered experimental fields, numerous small garden patches, and two private fields under constant observation during the early part of the summer, anthracnose was found only in the five experimental fields.

A diagram showing the weather conditions during this season is presented (fig. 12), to which reference may be made in connection with the discussion of the progress of the disease.

The disease was first noted on July 19, in field 1, planted five weeks previously. This center consisted of infection on two adjacent plants. As in 1915 the first infection was not found until after the rows had been thinned. All of the lesions were less than 1 cm. in diameter. On July 20 another center of two infected plants was found in field 1, and three centers of infection were found in field 2. On July 21 a third center was found in field 1 and a center was found in field 3. On July 22 anthracnose was found on one plant in field 4 and a center of two infected plants was found in field 5.

Rather thorough inspection of fields 1 and 2 made on July 26 and 27 revealed 12 additional centers in field 1 and two in field 2. On August 1 eight more centers were found in field 2 and the following day five more were found in field 1. The relation of this rather sudden increase in the number of new centers of infection to weather

conditions is not clear, unless the rains of July 12, 16, and 19 served to render conspicuous the incipient centers already existent.

We have then, on August 1, 20 centers in field 1 and 13 in field 2, none of which were showing much secondary infection. The further observation of anthracnose occurrence in fields 3, 4, and 5 was inter-

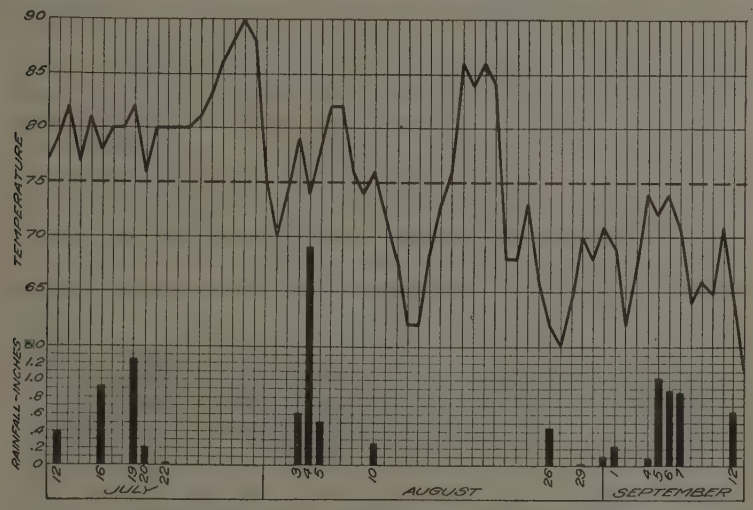


FIG. 12.—Diagram showing the weather conditions at Madison, Wis., during the summer of 1916. The curve represents the daily mean temperatures; the dotted line, the approximate optimum for the fungus; the black columns, the rainfall.

fered with by the removal of plants in connection with a mosaic control experiment. The data presented above, as well as results that were obtained later on these fields, are summarized in Table III.

TABLE III.—*Anthracnose in fields at Madison, Wis., in 1916.*

Field.	Date first noted.	Progress of disease.					
		Date.	Number of centers.	Date.	Number of centers.	Date.	Number of centers.
No. 1	July 19	July 27	15	Aug. 3	20	Aug. 15	46
No. 2	July 20	...do....	5	Aug. 1	13	Aug. 14	30
No. 3	July 21	July 21	1	July 26	5	Aug. 11	12
No. 4	July 22	July 22	1	.....	.....	Aug. 18	6
No. 5	...do....	...do....	1	.....	.....	...do....	10
No. 19	Aug. 24	Aug. 24	1	.....	.....	Aug. 30	2

The slowness of spread of the disease up to the first week in August may be in part explained by the extremely hot, dry weather of July, with no heavy rains after July 20. Reference to the weather chart will show that the daily mean temperatures during the latter part of July were well above the optimum (75° F.) for this fungus. Edger-

ton (14) finds the summer heat of Louisiana inhibiting bean anthracnose, and a similar situation may have existed in the case under consideration.

Very heavy rainfall on August 3, 4, and 5 was followed by a marked spread of the disease in fields 1 and 2. A rather careful inspection of field 1 on August 15 disclosed about 26 more or less distinct additional centers of infection, accompanied by rather widely scattered secondary infection in many cases. A similar situation obtained in field 2, and careful inspections on August 11 and 14 yielded a total of about 30 centers of infection, around most of which there was extensive secondary infection. This tended to extend farthest in the direction of the slope of the land and will be referred to later in connection with the consideration of water dissemination. On August 17 the disease was generally distributed in field 3 among the plants which had not been removed, especially at the lower corner of the field. A small private patch adjacent to this corner and hitherto undiseased, which received much of the drainage from field 3, also showed much infection of uniform age about this time. In field 4, occupying a very gentle slope, the disease did not become prevalent. By August 18 there were 10 centers in field 5 from which considerable spread had occurred, notably in the direction of the slope. Since an incubation period of a week or more is quite normal in the field, this first general spread of the disease seems to be closely associated with the period of abundant rainfall above mentioned.

During the week after August 18 the destructive effect of the disease became very apparent; the centers of infection were characterized by the death of the older diseased leaves and the rapid blighting of those near by. New centers of infection were found among the plantings of cucurbit varieties. Some new infection, due perhaps to the light rain of August 10, also appeared, but nowhere in such abundance as the infection which followed the heavy showers of the first of the month. Here it should be noted that, following this rain, the daily mean temperatures were quite low for five days. Occasional incipient lesions appearing within the infected areas previous to the rain of August 26 indicate that rain is not essential to infection. An hypothesis to account for the latter phenomenon is that, while rain is essential to the actual dissemination of the spores, germination and infection may occur later in the heavy dew which is formed during clear nights in August.

No anthracnose appeared in field 19, occupying level muck soil, until August 24, and it then remained confined to two small centers. In fields 9 and 10, belonging to private growers, no anthracnose appeared during this season.

Observations of August 21 to 31 made upon fields 1 and 2 showed that, as a rule, no marked extension of the areas of infection had

occurred since that resulting from the heavy rains of the first week in the month. The light showers of August 26 seemed to result in very little new infection, due possibly to the low temperatures following this rain.

Infection was sufficiently distributed and abundant, however, to serve as ample source for a general spread of the disease in fields 1 and 2 following the heavy rains of September 4 to 7. A general epiphytotic became evident on September 12 and 13, the lesions being more or less of a uniform age. In field 1 the disease was not only prevalent in practically all of the plats, but the cucurbit varieties and a series of interplanted cucumber seedlings, all at the foot of the general slope of the field, became generally diseased, quite evidently as a result of surface-drainage infection. While the frost of September 15 killed the vines, anthracnose was prevalent on the remaining cucumber fruits of all sizes, as well as upon the fruits of the susceptible cucurbit varieties, and on these fruits the disease persisted and even spread consistently during the remainder of the month.

It is to be noted that the disease first appeared, as in 1915, near the middle of July and that there were two periods of extensive spread following the two periods of heavy rainfall. There also seems to be a rather noticeable temperature correlation, since marked spread of the disease did not result from the rains followed by excessively high or rather low temperatures. Owing to the lateness of a killing frost, the latter of the two periods of spread above mentioned developed into a genuine epiphytotic, thus demonstrating the potential destructiveness of this disease.

DEVELOPMENT IN 1917.

Observations made during June, 1917, in the watermelon regions of the South indicate that here again the weather plays an important rôle and that in general anthracnose becomes widespread only late in the growing season of the crop. Original centers were found in the fields where fruits were only one-third grown, and in some fields infection was still limited to original centers when the fruits were mature. In Georgia, where drought had prevailed, anthracnose was not yet prevalent in the fields, but later, in Florida, after the rainy season had begun, anthracnose was found quite prevalent in the commercial fields, from which shipping had almost ceased, and especially in the immature seed crop.

At Norfolk, Va., the first week in July, the disease was found in the "original-center" stage in field cucumbers, while it had become widespread and destructive in some of the coldframe beds.

In 1917 at Madison, Wis., six cucumber fields were under constant observation. This summer was characterized by frequent rains and, except for the last half of July, rather low temperatures. Light rains

during the early part of July and a heavy rain on July 22, followed by temperatures favorable to this fungus, should have induced an early development of anthracnose. Although there were frequent rains during the remainder of the season, all daily mean temperatures remained well below 75° F., the optimum for the anthracnose fungus. The mean monthly temperature for August was 67° F., and for the first week of September, 63° F.

Anthracnose was first found on July 27 in field 1 and consisted at that time of eight infected plants scattered through four rows. On August 1 there was considerable anthracnose in 12 rows in the northwest corner of the field, in 3 rows in the southeast corner, and in a detached block at the southwest corner. This infection did not appear in typical original centers, but rather as scattered and comparatively recently infected plants. On August 13 anthracnose was found in a total of 28 rows scattered through the field, but was in no case serious. Inspections made August 25 and September 8 showed that no extensive spread had occurred. This can not be attributed to lack of rain and is probably due to the low daily mean temperatures prevailing after the end of July, as noted above.

In the other fields no anthracnose was found except in the Isom field, and there not until August 13, when old lesions were noted on the old leaves of two adjacent plants. On September 8 an additional original center from which very little spread had occurred was found. Why these were so late in developing is not understood.

LOCAL DISSEMINATION BY WATER AND OTHER AGENCIES.

Consideration of the subject of dissemination falls very naturally into two general categories: One, the mode of spread of the disease in the field during the growing season; the other, the manner in which the disease is first introduced into new fields (21). The former may conveniently be considered as local dissemination.

SPATTERING ACTION OF RAIN.

That water is essential to the separation and dispersal of the spores of this type of fungus is generally recognized. Since the spores tend to remain in masses adherent to the acervuli until their matrix is dissolved by water, the importance of the latter as a prerequisite for spore dispersal is quite evident. But the mechanical action of rain is more important.

The appearance of so-called centers in fields has been mentioned. The gradual enlargement of these foci of infection and the spread therefrom is the phase with which we are now concerned. Some of the experimental work on water dissemination has been previously reported (21).

Observations made in the Madison fields in 1916 showed that an enlargement of old centers of infection in all directions was to be

noted within five to seven days after each rain. A new "crop" of young lesions of uniform age, many within the old infected area in the row, many out around its periphery, appeared after each period of rainy weather. As these centers thus increased in size they extended not only along the row on either side but also across into neighboring rows and up hill as well as down. On the other hand, after a period of dry weather very little new infection was to be found. While this type of gradual spread of the disease may occur during light rains, it is, of course, more marked after heavy rains.

Considerable observational evidence relative to this question has been accumulated. In Princeton in 1915 abundant incipient infection was noted about old centers on July 26, probably as a result of the rains of July 17, 19, and 21. By August 9 and 10 a second crop of incipient lesions appeared, quite evidently as a result of the rains of August 1 to 5.

In 1916 an examination of the anthracnose centers in field 2 after a period of 10 days of dry weather revealed little or no incipient infection on new growth and only a few incipient lesions on old leaves. The latter may well be attributed to delayed germination or the penetration of spores actually disseminated during the earlier rains. Observations made in this field on August 8, 11, and 14 revealed abundant new infection as a result of the rains of August 3, 4, and 5.

It is commonly held that this type of spatter infection takes place by the actual spattering of the spores from diseased to healthy leaves. The agency of the soil as an intervening depository has not been emphasized. It seems quite likely that much of this spatter infection is accomplished by spores first washed to the soil in great numbers and thence splashed on to neighboring leaves.

To determine the validity of this hypothesis, a test was made of the soil under diseased leaves in field 2 five days after a light rain of August 10, 1916. Two grams of the soil was shaken up in 99 c. c. of sterile water and 1 c. c. of this suspension was transferred to a second 99 c. c. of sterile water. From the latter dilution 1 c. c. and fractions thereof were taken as inoculum for poured plates in water agar plus 2 per cent dextrose. Two soil samples were thus tested with five plates each. No colonies of the anthracnose fungus appeared.

On August 26, while the soil was still wet from a rain of 0.31 inch, two more soil samples were collected under diseased foliage and in a small drainage channel in field 2. Dilution plates were poured as above, four from each sample. In each set of plates six colonies of this fungus were identified. This indicated, according to the dilutions used, a spore content of 15,937 per gram of wet soil in one sample and of 60,000 per gram in the other.

To further test the possibility of leaf infection from soil, a large sample of soil was collected from anthracnose centers in field 2 on August 14, 1916, four days after a rain. Under healthy runners in each of eight locations in four plats in field 1, 4 tablespoonfuls of this soil were sprinkled. In an examination of these runners on August 31 infection was found in one case consisting of lesions on six leaves, abundant on three. In this instance the soil inoculum had been sprinkled upon the leaves and then shaken off, while in all the other tests an effort was made not to sprinkle the soil on the leaves.

These tests prove that the spores of this fungus are abundant in the soil under diseased plants immediately after a rain and are viable even four days later. The common occurrence of anthracnose lesions on the lower side of a watermelon fruit in the field indicates infection from spores in the soil or in drainage water.

With regard to the spattering of spores upon healthy leaves, two tests were made. Within two hours after a heavy rain, September 5, 1916, an undiseased leaf was taken from field 2 near diseased leaves and the blade washed in 200 c. c. of sterile water. From the five dilution plates poured from this wash water, one colony of anthracnose was isolated. This indicated, according to the quantity of wash water represented, that there were 96 spores on the leaf lamina. A similar test made with an undiseased seedling within 1 foot of diseased leaves yielded negative results. The isolation of the fungus from an undiseased leaf proves that the spores were present on the leaf surface after a rain.

During both seasons of 1915 and 1916 anthracnose occurred on plants under cheesecloth cages which eliminated both insects and pickers as agents of dissemination. Since these plants were very evidently not original centers of infection, the entrance of the fungus can be attributed only to water splashing through the cloth or, more likely, washing under the cages.

Among watermelons in the field, cases have been noted in which the fruit lesions were arranged in vertical rows in such a way as to indicate beyond a doubt drip infection from overhanging diseased leaves.

ARTIFICIAL WATERING.

Another line of evidence relative to the local spread of the disease by the spattering of water is furnished by the severity of anthracnose as noted among certain of the fields of cucumbers in coldframes at Norfolk, Va., in 1917. Here, daily watering by an overhead system was practiced, and this may explain in part the advanced development of the disease in these fields as compared with its relative obscurity in other fields of the region.

SURFACE DRAINAGE WATER.

But the mere spattering of rain and blowing of the droplets by wind can not account for the extensive epiphytotics of this disease which suddenly appear in certain fields. A considerable mass of observational evidence has been accumulated relative to this point. It has been generally noted that extensive spread of this disease does not follow every rain, but only the very heavy rains during which considerable surface run-off takes place. The general trend of the evidence seems to indicate that epiphytotics tend to occur more commonly where the topography of the field is sloping or rolling.

As to the effect of heavy rains, it will be recalled that the first general spread of the disease in the Princeton fields in 1915 followed the rains of the first week in August. In the Madison fields in 1916, two periods of extensive spread of the disease were clearly recognized, one after the heavy rains of August 3 to 5 and the other, which resulted in a serious epiphytotic, after the heavy rains of September 4 to 7. Intervening lighter rains of 0.28 inch on August 10 and 0.31 inch on August 26 were not followed by an extensive spread of the disease. It is realized that here there is a difference in duration as well as in amount, and the longer rainy periods no doubt afforded conditions more conducive to infection. Furthermore, reference has been made to the significance of the lower temperatures of the periods following the light rains.

Additional observational evidence relative to the agency of surface drainage during heavy rains in spreading this disease is furnished by the direction of this spread from the old centers. This is revealed by the location of even-aged new infection following such rains.

Field 2 at Madison in 1916 afforded a good opportunity to observe this phenomenon. This field occupied a decided south slope and the rows extended across the slope. The new infection resulting from the rains of the first week in August was very evident by August 12 and a careful inspection of the field showed the manner of spread from the old centers of infection to be somewhat as illustrated in figure 13. There was a decided tendency for the greatest spread from the old centers to be across the rows and distinctly in the direction of the slope. At least nine of the areas of infection show elongations extending downhill; in fact, in four of these cases the new infection in the row below the old center was along small gullies or drainage channels leading directly from the old center in the row above.

Field 3 occupied a gentle southeast slope, and here again the rows crossed the slope. One original center occurred in a row along the upper edge, and from this center a drainage channel led down across the entire field to the southeast corner, where it formed a delta

extending into a private cucumber patch. On August 17 the course of this drainage channel was found to be marked by new infection in at least three rows and by a second old center. There was also much scattered new infection in the region of the delta, both in field 3 and in the private patch as well.

In field 4 the rows were parallel to the very slight western slope. On August 18 a spread of even-aged secondary infection to the westward from four of the six centers was noted. Examination of the anthracnose centers in field 5 on August 18 showed a downhill spread of infection across the rows in three instances. In two cases

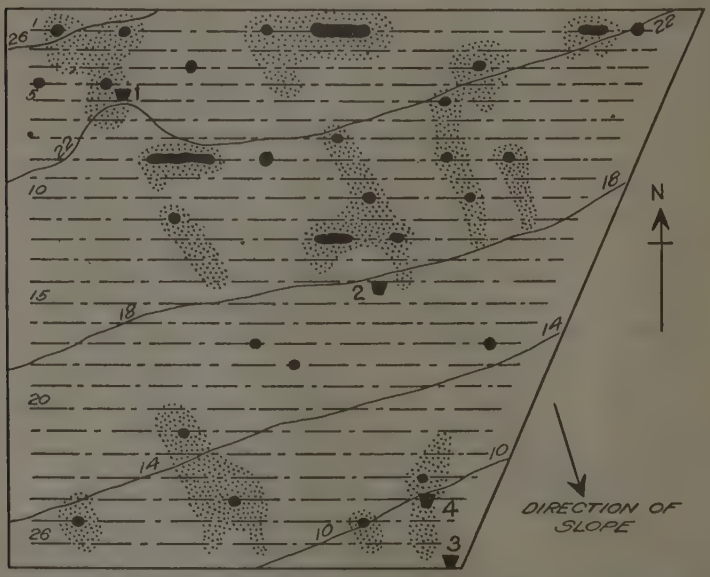


FIG. 13.—Diagram of field 2, Madison, Wis., August, 1916, showing the relation of surface drainage to the distribution of anthracnose. The cucumber rows are indicated by broken lines and the slope by the 4-foot contour intervals. The four trapezoids represent water traps 1 to 4; the black circles and ellipses, original centers of anthracnose; and the shaded areas, new infection noted on August 12.

this infection extended across two rows downhill (14 feet), and at the same time into the adjacent row above.

Among the Madison fields that season, anthracnose became prevalent only where there were slopes. In field 19 on level muck soil, there was very little spread from the old centers. In a large watermelon field near Quitman, Ga., infection was found to be widespread on certain slopes, but still restricted to small areas in the more level parts.

Late in the season of 1916 an excellent opportunity was afforded in field 1 to observe the results of the heavy rainfall of the first week

in September. As may be noted in the accompanying diagram (fig. 14), this field occupied a decided westward slope, and the rows were parallel to the direction of the slope. Along the west side at the foot of the slope were grown the various cucurbits mentioned in the discussion of pathogenicity. While some anthracnose appeared among these earlier in the season, it was confined to a few well-defined centers. After the rains mentioned above, new even-aged infection of anthracnose was found on September 12 on all of the rows

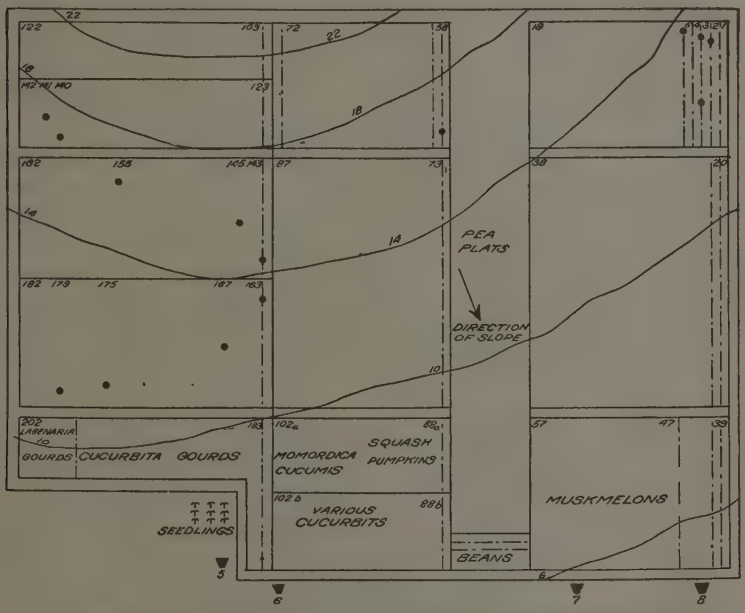


FIG. 14.—Diagram of field 1, Madison, Wis., 1916. The direction of the rows is indicated by broken lines, the slope by the 4-foot contour intervals. Where not otherwise indicated the crop was cucumbers. The original centers of anthracnose noted on July 27 are shown by the circles. The location of the seedlings planted late in the summer is shown in the lower left corner. Water traps 5 to 8 are shown by trapezoids.

of susceptible cucurbits, very evidently as a result of surface drainage from the diseased field above.

Still more convincing evidence was furnished by rows of cucumber seedlings planted late in the season between the rows in field 2 and on the west edge of field 1 (fig. 14). On September 11 these seedlings in field 1 appeared healthy, while the next day practically all of them were thickly spotted with incipient anthracnose lesions, so numerous as to cause many leaves to curl. By the next day many seedlings were dying. Drainage channels from the field above led directly through these rows of seedlings. Very evidently these seedlings were so abundantly infected during the preceding rains that they were practically blighted outright by the numerous lesions that developed simultaneously. An added point of interest is that this

drainage water also passed through the rows of Cucurbita gourds, none of which became diseased (fig. 14).

In field 2 all of the rows of interplanted seedlings showed recent and even-aged infection on September 13. Besides leaf and cotyledon lesions numerous enough to cause these organs to curl up, some seedlings bore girdling stem lesions as well. This infection is likewise attributed to surface drainage during the rains of the week previous.

That the spores are washed from the leaves to the soil was proved by the tests previously cited. That the spores are carried considerable distances by the surface run-off during rains and spattered from this drainage water upon all plants along the drainage channel is to be inferred from the observations presented above.

To test experimentally the latter hypothesis, steps were taken to prove that the spores were present in surface drainage water. On August 14 four glass tumblers were sunk flush with the soil at the points indicated in figure 13, so as to intercept surface drainage water during the next rain. No tests were made until the rains of the first week in September. On the morning of September 5 the traps were emptied. A heavy shower occurred in the afternoon, after which the contents of the four traps were collected in sterile flasks. At the same time samples of the soil near two of the traps were collected.

Each sample of drainage water was tested by poured plates as follows: Of the drainage water 1 c. c. was transferred to a flask containing 99 c. c. of sterile water, and from this 1 c. c. was transferred to a second 99 c. c. flask. Using recorded amounts of inoculum from these dilutions, three plates were poured from the first flask and two from the second in water agar plus 2 per cent dextrose. No colonies of the anthracnose fungus appeared in any of these plates. The soil samples were also tested by the method outlined earlier, and only negative results were secured.

That spores of the anthracnose fungus were present in the samples of drainage water was proved in another way, however. The contents of each trap, after the removal of the sample for plating, were sprayed upon healthy potted cucumber plants.[1] The results of these tests are presented in Table IV.

Spores of the anthracnose fungus were present, therefore, in three of the four traps. Trap 1, in which no spores were found, was located near the upper edge of the field, and hence there was a smaller reservoir of disease contributory to it.

After the additional rains of September 6 and 7 a series of five plates each was poured from traps 1 and 2 in field 2, and, as in the

[1] These inoculations, as well as those described later, were made by Dr. E. Carsner in connection with his investigation of the angular leaf-spot of cucumber.

previous test, only negative results were obtained. Owing to the ravages of mosaic, field 2 was becoming unsuitable for work on anthracnose and field 1 offered better possibilities.

TABLE IV.—*Anthracnose dissemination test by spraying potted cucumber plants with drainage water from water traps.*

Trap.	Serial numbers of pots sprayed on Sept. 5.	Number of anthracnose lesions on Sept. 14.	Trap.	Serial numbers of pots sprayed on Sept. 5.	Number of anthracnose lesions on Sept. 14.
No. 1	139	None.	No. 3	136	5
	148	None.		137	None.
No. 2	145	2	No. 4	140	7
	146	1		138	6

On September 12 four water traps (5, 6, 7, and 8), similar to those in field 2, were placed in field 1 along the extreme western edge and at the foot of the slope. The location of these is shown in figure 14. The same day a rain of 0.66 inch occurred and the contents of these traps were collected immediately afterwards.[1] Using the method outlined above, dilutions were made from three of the samples and a series of seven plates poured in each case. No anthracnose colonies appeared in the plates from traps 5 and 7, but in the series from trap 6 two colonies of the anthracnose fungus appeared. According to the dilution used, this result indicates a spore content of 916 per c. c. in the water in trap 6.

The liquid collected from these traps was also sprinkled on healthy potted plants in the greenhouse.[1] Seven pots were thus inoculated, and on September 19 the plants in each pot were diseased. Each sample produced anthracnose infection, and it is quite evident that spores were present in the drainage water caught in each trap. It is also apparent that this method is superior to the poured-plate method for detecting the presence of the spores in such an inoculum.

These experimental tests prove conclusively that which the observational evidence indicated, namely, that the spores are carried through the fields by surface drainage water during rains. As to the distance carried, recovery of the fungus from these traps along the edge of the field indicates that the spores were transported at least as far as the extremities of the field in question, and there is no reason to suppose that they might not be carried much farther. However, the main importance attached to this agency is that it serves to disperse the fungus far and wide through the particular field already containing original centers of infection.

Water, then, is not only necessary for the germination of the spores, but is also essential to the separation of the spores from the lesion. In the shape of rain, it washes the spores to the soil and spatters them on to the host plants. In the shape of surface run-off, it dis-

[1] These traps were placed and the contents collected by Dr. E. Carsner.

perses the spores widely through the field and causes a marked downhill spread of infection.

MOISTURE CONDITIONS FAVORING INFECTION.

Judging from the evidence already presented under the consideration of water dissemination, it seems quite evident that the very agency of dissemination in that case furnishes the proper condition for spore germination and subsequent host infection. In view of the even-aged "crops" of new lesions following each rain, it appears that infection with anthracnose generally occurs while the plants are still wet from a rain.

However, it was pointed out that some incipient infections are often to be found after periods of prolonged drought, and this was attributed to delayed penetration by spores already present. Whether these spores were present as such or as appressoria is not known, although it seems quite probable that the latter may represent a resistant resting stage.

Now, as to the conditions favoring such cases of delayed infection, it is of importance to recognize that during August in Wisconsin heavy dews occur at night and cucumber foliage is heavily coated with moisture. Observation has shown that this water of condensation remains in drops on the lower epidermis of cucumber leaves, while it spreads over and thoroughly wets the upper epidermis. This daily wetting of the leaves no doubt affords favorable conditions for germination of the spores or penetration from the appressoria.

Furthermore, there is a possibility that infection may be facilitated by guttation water. Large drops of guttation water often collect about the margins of cucumber leaves when evaporation is retarded, as, for example, in the case of plants under cloth cages. This water is sometimes exuded in such quantities as to run down over the epidermis of the leaf. Marginal anthracnose lesions are common. Two samples of guttation water were collected from caged plants, September 6, 1916, and tested as a medium for spore germination. While only a very low percentage of germination was obtained, proof was afforded that the liquid was not toxic.

Examination of watermelon fruits in the South showed the frequent occurrence of dense masses of incipient anthracnose lesions, or "pimples," about the periphery of the bleached area where the melon was in contact with the soil in the field. Subsequent examination of fruits as they lay in the field revealed that this region of their surface was coated with a layer of minute droplets of condensation water, while the part in actual contact with the soil was thoroughly wetted. This condition obtained even during the heat of the day. It seems quite probable that the exposed band of condensation moisture on the under side of the fruit would afford conditions of moisture and oxygen supply very favorable to spore germination.

While rain water may be the usual requisite for infection, it is evident that other conditions may also permit its occurrence.

DISSEMINATION BY INSECTS.

While insects may possibly act as agents of dissemination, there is no indication that they are at all important in this capacity. It has been previously noted that the striped cucumber beetles often feed on the tissue about the margins of leaf lesions, and it is evident that infectious material might be subsequently transported by these beetles.

A few tests were made to determine what part insects might play as agents of disease transport. On August 11, 12 striped and 12 spotted beetles were collected from anthracnose centers in field 2 and about 10 of each species were introduced under two cheesecloth cages covering plants in field 1. Drainage-water infection of the plants under the cages in this field ruined this test. On September 6 three spotted beetles were collected in sterile test tubes from diseased plants and each beetle was placed in 50 c. c. of sterile water. Plates poured from this wash water yielded no colonies of the anthracnose fungus.

No proof was obtained, therefore, that insects spread the disease, and observational evidence has not tended to implicate them.

DISSEMINATION BY CULTURAL PRACTICES.

Since the spores may occur abundantly in the soil, it seems quite possible that the disease might be spread during the processes of cultivation. For this disease, unlike bean anthracnose, observation has not yielded indications of this type of dispersal.

The process of picking the cucumber crop grown for pickles would seem to afford a very fruitful means of disease dispersal. Since this process is repeated at least four times a week and involves considerable handling of the vines, it may contribute much to the spread of the anthracnose along the row from an old center. This is especially true if picking is done when the vines are wet with dew or rain. At one of the coldframe farms near Norfolk where the disease was very serious, it was found that picking was done early each morning, when, no doubt, dew was on the vines.

The occurrence of more or less isolated leaf lesions here and there among the leaves is taken as evidence of this type of dissemination. From these lesions as a source, new centers of infection may develop. Such infection differs from the type produced by drainage transport in that, in the latter, the infection is continuous along the row from the old center.

Among watermelons, quite conclusive observational evidence was secured relative to the agency of man in spreading anthracnose. In a

large field near Quitman, Ga., considerable new infection in the shape of rather isolated infected leaves or groups of leaves was found out along the rows just outside of extensive heavily infected areas. These new infections were not downhill from the old centers nor was infection continuous along the row, so water dissemination was precluded. As a rule, these infections consisted of only a few older lesions, not scattered over the whole leaf blade, but commonly along one edge or along a vein. From these older lesions some secondary infection had occurred. The most striking feature of these incipient centers of infection was, however, that about two out of three were located on the leaves immediately surrounding or overhanging a fruit. About the edge of one area of old infection, 68 of these scattered new infections were found on foliage around fruits. This represented probably 5 to 10 per cent of all fruits lying within the area examined.

Upon inquiry of the plantation manager, it was learned that about two weeks previously the field had been gone over for the purpose of removing the malformed and rotting fruits. This period of time coincided well with the apparent age of the older leaf lesions above noted. This "culling" operation, it appears, is usually performed early in the morning when the vines are wet with dew. At any rate, in the case under consideration, all the evidence pointed to hand dissemination during the process of culling the vines. A likely hypothesis is that, in going up one row and down the next, the workman had repeatedly passed through the badly diseased area, had removed diseased fruits, and carrying spores on his hands had unconsciously inoculated the foliage farther along as he pushed the leaves aside to gain an unobstructed view of a fruit or to see if a fruit was concealed under the heavy foliage.

From these leaf lesions, spores are readily washed to the fruit underneath, and the insidiousness of these "hand" infections becomes obvious when it is realized that the new centers are so located that the worst possible damage is an immediate result.

The chief activity of man in local dissemination is, then, in inoculating leaves here and there along the row, which act as new centers of infection. Among watermelons, rather conclusive evidence of this type of disease dispersal has been obtained.

DISSEMINATION WITH THE SEED.

It is in the introduction of the disease into new fields that human agencies assume prime importance. Here will be considered some of the possible sources of infection. The overwintering of the fungus in the field is treated in a later chapter. The disease has not been found on wild cucurbits in the North. The occurrence of Lagenaria gourds and volunteer melon vines in the South is recognized as a possible source of infection. Country-wide transport of diseased water-

melons unquestionably is another means of long-distance spread of the disease provided infectious material reaches the fields.

It seems fairly safe, however, to eliminate all of the above factors except overwintering as rather remote possibilities. As an explanation of the appearance of the disease in new localities, all evidence points toward introduction with the seed. Previous workers have suggested this possibility, notably Sheldon (46; p. 127–137), Garman 22, p. 51), Eckardt (11), and Eriksson (16, p. 126–127). Eriksson presents observational evidence which strongly indicates the introduction of anthracnose into greenhouses in Sweden with cucumber seed from England.

FIELD OBSERVATIONS.

The experimental field at Princeton in 1915 had previously been in sod for seven years. Anthracnose appeared in this field as soon as in any other fields of the region. So far as could be learned none of the five experimental fields at Madison in 1916 had previously grown host crops, yet the disease appeared simultaneously in all of these fields and in practically no other fields or gardens of the surrounding territory. In the watermelon industry of the South crop rotation is necessitated by the ravages of the wilt, yet anthracnose recurs annually.

Instances could be multiplied, but sufficient evidence has been adduced to show that the disease appears quite commonly in new fields. In a case such as that presented by the 1916 Madison fields there seems to be but one plausible explanation, namely, disease introduction with the seed.

The very manner in which the disease originated in these fields suggests this hypothesis. The disease first appeared in the Princeton field and in the Madison fields in what we have termed "original centers." These were scattered here and there through a field and were usually limited to a comparatively small number per acre (figs. 13 and 14). With a disease as infectious as this it is not always easy to differentiate between original and secondary centers, but in fields under constant observation those centers appearing first and about simultaneously may safely be termed "original."

The nature of these initial infections furnishes another argument in favor of their origin in situ. The first original center found in the Princeton field consisted of two adjacent plants not yet large enough to "run." These plants bore lesions on the first and second foliage leaves. Since the rows had already been thinned, several plants had been removed between and adjacent to the two above noted, and the hypothetical originally diseased plant might already have been removed.

In 1916 the first center of anthracnose found in field 1 consisted of two adjacent plants, one bearing one lesion on the fourth leaf, the

other bearing one lesion near the tip of a cotyledon, one lesion on the first leaf, two on the second, and four on the third leaf. Unfortunately, thinning had again been completed. The next day in another part of the field an infected plant was found which had three lesions on the first leaf but none on the cotyledons. The same day two other centers of two infected plants each were found and the next day two more similar centers were found. In all of these cases the lesions were fairly large, indicating that infection had occurred perhaps two weeks previously.

In the other four fields, similar original centers of apparently the same age were found at about the same time. In field 2, the first one found consisted of two adjacent plants, one with numerous lesions on the first leaf. The first original center found in field 3 also consisted of two diseased plants, of which one bore lesions on the first leaf and on a cotyledon. In field 4 the first anthracnose center was one very badly spotted plant, and in field 5 the first center noted consisted of two diseased plants. Whether or not the originally diseased plant was present in any of these cases is rendered questionable because of the previous thinning operation. Just how the fungus had passed the five weeks elapsing since the time of planting the seed can not be answered at present.

Near Albany, Ga., in a large watermelon field not previously planted with this crop, careful search of about 1,000 hills revealed only two single-hill centers of anthracnose. In each of three melon fields near Monticello, Fla., one single plant center of anthracnose was found. Single plant centers of anthracnose were found in one field of cucumbers near Norfolk, Va.

Under the fairly well controlled conditions among the Madison fields in 1916, the simultaneous appearance of a few scattered centers in each of the five experimental fields on land not previously sown to this crop furnishes quite convincing evidence of disease introduction with seed. But still more striking is the fact that anthracnose appeared only in the five fields planted with seed from the same source and not in the two private fields and numerous gardens also under close observation (21). This correlates the occurrence of disease with seed from a particular source.

ANTHRACNOSE IN SEED FIELDS.

In view of the probability of seed carriage of the disease, as indicated above, the next step was to ascertain what opportunity there was for the seed to become contaminated. First, was the disease present in the seed fields?

A visit was made the first week in October, 1916, to a seed farm in Ohio. The vines were dead at this time, but anthracnose was found very prevalent on the fruits in certain fields. Since the seed fruits

are allowed to lie in the field until thoroughly ripe, there is abundant chance for their infection. The disease was also found on cucumbers in several of the fields on a seed farm in Michigan, but it was not present in the melon fields. In August, 1917, the disease was found to be rather serious in several fields of cucumbers on another seed farm in Michigan, and in October, during the seed-harvesting season, anthracnose was found very prevalent on the seed fruits in one field. The badly diseased condition of the fruits in one hill is shown in Plate VII and the lesions on a seed fruit in Plate VIII.

On June 29, 1917, 12 seed fields were visited in Florida in the chief watermelon seed-producing region. Anthracnose was found in six of these fields; in one it was very serious and widespread and occurred on the young fruits as well as on the vines. The melon seed crop is later than the commercial crop of that region and runs along well into the rainy season of July, so that anthracnose development is favored. Melon seed is also secured in other districts from the unsalable fruits remaining in commercial fields after the better ones are shipped. This almost insures the use of diseased fruits. In any case, the melons are allowed to remain in the field until dead ripe, and on account of the higher temperatures of that region they are more subject to severe anthracnose infection than seed cucumbers.

Although the disease has been found in the State, no anthracnose has been reported in the cucurbit-seed fields in Colorado, and since practically all cantaloupe seed is produced in the West, this may explain the absence of anthracnose from the cantaloupe fields as observed at Blackville, S. C.

It is quite evident, then, that the disease is present in both cucumber and melon seed fields and that seed fruits become abundantly infected. It remains to be shown whether or not seed infection or contamination may occur. Theoretically, the fungus might be present within the seed, upon the surface of the seed, or in diseased fruit fragments mixed with the seed. First, what is the likelihood of the two latter contingencies?

PROCESSES OF SEED EXTRACTION.

In the North, cucumber and melon seed is extracted by passing the fruits through a machine known as a "grinder." The fruits are thrown into a hopper and pass down between revolving cylinders, the surface of which is spiked, ridged, or fluted. These rollers crush the fruits sufficiently to free the seed and pulp. The wet mass of seed, pulp, and rind fragments then passes through inclined revolving screens, which separate the seed and juice from the larger fragments of rind and pulp. The fluid mass of seed and juice is usually allowed to ferment two to four days in open barrels or pits, to remove from the seed the adhering pulp and the capsule or gelatinous epidermal

layer of the seed. After this, the seed is thoroughly washed in a screen and dried in flats in the open or in a drying room. In the case of fermentation in pits, the seed may be dried without washing. By some growers, the fermentation process is now omitted.

During the process of crushing and separating, the exterior of the rind becomes thoroughly drenched with the abundant juice from the crushed fruits, and it is inevitable that the spores should be washed from the fruit lesions into the juice which goes through with the seed. Conditions at the Ohio farm were very interesting in this respect. A considerable percentage of the fruits being ground bore anthracnose lesions, and, although the latter were not sporulating to any great extent, there appeared to be plenty of opportunity for wholesale contamination of the seed by the spores of the fungus. Similar conditions prevailed in the Michigan seed field visited in 1917, where a much larger percentage of the seed fruits was diseased.

In an attempt to prove that anthracnose spores were present in the seed and juice as it issued from the grinder and in the liquid in fermenting barrels filled the same morning, cultural tests were made at the Ohio farm. Test-tube water blanks were used in place of dilution flasks and sterile calibrated pipette droppers were used in place of pipettes. Two series of six plates each were poured, using water agar plus 2 per cent dextrose. No anthracnose colonies appeared. The heavy seeding of bacterial and fungous colonies in these plates showed, however, that the liquid tested contained an abundant and varied flora.

On October 6, 1917, a number of diseased seed cucumbers, one of which is shown in Plate VIII, were collected at a seed farm and brought to the laboratory, where the seeds were removed by hand the next day. To simulate as closely as possible the conditions in the commercial operation, the seeds, juice, and rinds were mixed together in a jar and then the larger rind fragments were removed. The material in the jar was allowed to ferment for two days in the laboratory. The gas produced yielded a froth which buoyed up numerous rind fragments. In such diseased fragments thus caught and held at the surface the fungus was found to be producing new acervuli and sporulating in abundance, thus greatly increasing the amount of infective material in the liquid.

The period of fermentation might allow the spores to germinate, but it is probable that the anaerobic conditions would prevent this. In case germination did occur on the wet seed, adherent appressoria would quite likely result. It is not considered at all likely that the biological action during this short period of fermentation would kill the spores. The washing process would remove by no means all of the spores from the seed, since the surface of the latter is not smooth but is covered with cellulose hairs. During the process of drying in

ANTHRACNOSE ON SEED CUCUMBERS IN THE FIELD, OCTOBER 6, 1917, JUST PREVIOUS TO SEED EXTRACTION.

ANTHRACNOSE LESIONS ON A SEED CUCUMBER, OCTOBER 6, 1917.

the open, exposure to sunlight might kill a negligible percentage of the spores. There remains only the factor of actual desiccation, which must be endured by every spore. The spores are, however, quite resistant to desiccation, and as to their longevity while in this state more will be said presently. The subsequent processes of rewashing, floating out the light seed, redrying, and fanning to remove skin fragments are not such as to eliminate contamination.

Furthermore, not all of the fragments of fruit epidermis and pulp are removed. A considerable percentage of the Ohio seed bore such adhering fragments, and it is quite possible that infectious matter may remain on the seed in the shape of mycelium within these fragments. In a small sample of unfanned seed from the seed field observed in October, 1917, numerous fragments of fruit epidermis dotted with blackened acervuli of this fungus were found both free and adherent to seed. In an attempt to prove the viability of the spores on these acervuli and of the mycelium within the tissue by plate tests on December 20 success was not obtained.

In the watermelon-seed regions some of the growers operate machines, but by the majority the work of seed extraction is done by hand. The fruits are cut in two and the seed and pulp scooped out by hand into tubs, where the mass is allowed to ferment. Then the seed is washed and dried. During this operation infectious material from surface lesions may readily gain access to the seed.

The process of seed extraction among cucumbers and melons therefore affords ample opportunity for seed contamination with infectious material, and no step in the whole process precludes the possibility of the latter remaining viable and going into storage with the seed.

FACTORS INFLUENCING EXTERNAL SEED CARRIAGE OF THE FUNGUS.

There still remains the long period of desiccation upon the seed surface to be reckoned with. Much of the melon seed used is 2 or more years old, and this fact offers a real difficulty. But much of the cucumber seed, for pickles at least, is used the next season and is in storage only seven to eight months. In this regard it should be recorded that spores on leaf lesions in dried specimens have commonly been found viable seven months after the collection of the material. Many spores should therefore remain viable on the seed until the next season, and were appressoria present the resistance to desiccation might be still greater.

It is also important in this connection to understand the nature of the seed surface. The familiar gelatinous capsule of freshly extracted cucumber seeds is the epidermis of the seed and consists of a single palisade layer of extremely elongated columnar cells prismatic in cross section and 160 to 260 μ or more in height (1, p. 283–

286). The lateral walls are thin except for a rodlike thickening extending from base to apex of each cell. The processes of fermentation and washing remove all of this cell layer except these rodlike thickenings, most of which remain as cellulose hairs (fig. 15). These hairs become closely appressed to the seed when the latter is dry and may protect adhering spores or appressoria from extreme desiccation. Furthermore, since the fungus may be present in the adhering fragments of fruit tissue it is evident that in this form desiccation could be more readily endured.

It is scarcely believed at present that the fungus will survive an additional year's storage, and hence it is only reasonable to maintain that the disease is introduced only with seed planted the year after it is harvested. This is an important consideration, since in practice much of the cucumber and melon seed is more than a year old when used. Cucumber seed is known to remain viable from two to six years, and many growers prefer old seed.

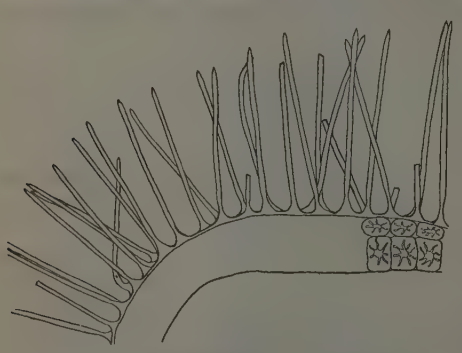


FIG. 15.—Cross section through the seed coat of a cucumber seed, showing cellulose rods, or "hairs."

POSSIBILITY OF INTERNAL CARRIAGE OF MYCELIUM.

There is also the possibility of internal carriage of dormant mycelium within the seed. Eriksson (16, p. 127), after failing to find mycelium in supposedly infected seed, advances his mycoplasm theory to account for anthracnose carriage in cucumber seed. The depth of fruit lesions as observed on seed cucumbers and watermelons suggests that seed infection may occur previous to seed extraction. From observations made upon seed cucumbers collected in October, 1917, it appears that there may be considerable opportunity for the internal infection of seed. Coalescent lesions were found penetrating the placentæ as far as the seed, and in some instances the gelatinous capsules were decomposed and the funiculi rotted off at the points of attachment to the seeds.

Samples of seed removed from beneath such lesions were tested in two ways for the presence of the anthracnose fungus. The seeds were sterilized in mercuric chlorid, 1 to 1,000, for five minutes, washed in sterile water, and some were planted intact in poured plates of 2 per cent dextrose agar. From others the embryos were aseptically removed and planted in similar plates. Tests were made on November 4 and December 14. In 87 seeds tested by the former method and 90 by the latter no trace of the anthracnose fungus was obtained.

ATTEMPTS TO PROVE THE PRESENCE OF THE FUNGUS ON THE SEED.

With all of the observational evidence pointing so strongly toward seed carriage of the fungus, repeated efforts were made during the spring of 1917 to isolate the organism from commercial seed or in other ways to prove its presence.

Besides numerous samples of seed collected at the Ohio farm at different stages in the cleaning process, a generous supply of 1916 seed was later secured from that farm. This seed, as well as some of that used in 1916, was tested in a variety of ways in an attempt to detect the presence of the anthracnose fungus. The following methods were employed:

(1) Seeds were placed in agar plates.

(2) Seeds were planted in Petri dishes on sterile filter paper moistened with water or a nutrient solution.

(3) Seeds were planted in large damp chambers on moistened filter paper.

(4) Seeds were planted in sterile sand in damp chambers.

(5) Seeds were planted in sterile soil in flats in the greenhouse.

In the first three methods the fungus might be detected by its saprophytic growth on the substratum or by its parasitic development on the seedling. In the last two methods the latter possibility was relied upon. The results of these tests are summarized in Table V.

TABLE V.—*Summary of laboratory tests of cucumber and muskmelon seeds to prove the presence of the anthracnose fungus.*

Date.	Seed used.		Method.	Time in days.
	Source.	Number.		
1916.				
Aug. 28	Iowa, 1915	(1)	11 dilution plates in 2 per cent dextrose agar from wash water.	8
1917				
Feb. 5	Ohio	15	5 in each of 3 plates, 2 per cent dextrose agar	8
5	Muskmelon seed, 1915	15	....do	8
5	Ohio (fruit tissue adhering)	24	6 in each of 3 plates, 2 per cent dextrose solution in filter paper.	17
5	Ohio	8	Same, in plate	17
5	Ohio (fruit tissue adhering)	30	6 seeds in each plate; filter paper plus water	17
26	Ohio	200	Wash water on cucumber plants	14
26	Ohio (fruit tissue adhering)	33	In drops of water on cucumber foliage; 2 plants.	(2)
Mar. 3	Ohio	1,200	12 sterilized sand flats, in greenhouse	14
12	....do	150	Damp chamber, wet filter paper	18
12	Ohio, collected in October, 1916.	150	....do	18
13	....do	200	Sterile sand, in damp chamber	23
14	....do	48	2 per cent dextrose solution, in filter paper	16
23	Ohio	6	Wet filter paper	16
23	....do	12	Prune decoction, in filter paper	16
23	Iowa, 1915	6	2 per cent dextrose solution, in filter paper	15
23	....do	6	Wet filter paper	15
23	....do	12	Prune decoction, in filter paper	15
23	Ohio	100	Wet filter paper, in damp chamber	17
24	Iowa, 1915	100	....do	16
27	....do	100	Sterile sand, in flat	16
27	Ohio, collected in October, 1916.	100	....do	16
27	Ohio	1,100	....do	16
27	....do	400	Sterile loam, in flat	16
May 1	Ohio (fruit fragments adhering).	60	Sterile sand, in damp chamber	14
	Total	4,069		

[1] Two pounds.

[2] Failure.

In a total of more than 4,000 seeds tested no anthracnose was found. From the 3,100 seeds tested in sterile soil no diseased seedlings developed. Where culture media were used the profuse development of bacteria and fungi upon and around each seed proved that the latter bore an abundant and varied surface flora. On old seed the flora was less varied. The rapid development of these forms rendered anthracnose isolation by cultural methods a matter of great difficulty.

To determine whether or not the methods employed above were reliable, tests were made with seed previously dipped in a spore suspension. The results of these tests warrant a more detailed account. On March 13 about 500 seeds were immersed for 50 minutes in a heavy suspension of spores of the anthracnose fungus and then dried on filter paper. The next day 50 of these seeds were planted in sterile moist sand in a damp chamber. The seedlings were sprayed from time to time with sterile water. Sixteen days after planting, one seedling, which had carried its seed coat up with the cotyledons, showed an anthracnose lesion on the stem near the ground line and another on a cotyledon. Owing to the stem lesion the seedling had already fallen over, as in damping-off. On the stem lesion were numerous sporulating acervuli. In 22 days after planting, three more seedlings had damped-off with anthracnose and four others bore lesions at the ground line. In 29 days after planting, 16 out of the 45 seedlings had succumbed to anthracnose.

These results corroborate those secured by Carsner,[1] who obtained damping-off of cucumber and muskmelon seedlings when seeds previously dipped in a spore suspension were planted in sterile soil. Such results suggest that perhaps in the field the originally diseased plant may damp-off and disappear, so that the first lesions discovered are the result of spores splashed from this plant upon those adjacent to it.

To test the filter-paper culture method, 28 of these inoculated seeds were planted in four sterile Petri dishes on filter paper moistened with 2 per cent dextrose solution. Six days later, acervuli of anthracnose were found on two seed coats, and by nine days one more seed bore acervuli and its seedling was diseased. Two weeks after planting, two more seedlings were diseased. Mold and bacterial growth was as usual abundant on these seeds. The results of these and further tests with these seeds are presented in Table VI.

While it is evident that diseased seedlings may result from inoculated seed, it is noteworthy that not all inoculated seeds yield diseased seedlings in soil. Owing largely to the profusion of other organisms on the seeds, the methods of anthracnose detection used with the commercial seed, as summarized in Table V, show a low efficiency when applied to seed known to be heavily inoculated with the fungus.

[1] Carsner, E. Op. cit.

TABLE VI.—*Laboratory tests of seed inoculated with spores of the anthracnose fungus.*

Date of test, 1917.	Number of seed.	Medium used.	Days.	Result.
Mar. 14	50	Sterile sand, in damp chamber	29	16 out of 45 seedlings diseased.
14	28	2 per cent dextrose solution, in filter paper.	14	Anthracnose detected on five.
15	6	2 per cent dextrose agar	15	No anthracnose.
23	6	Wet filter paper	13	1 seedling diseased at heel.
23	6	2 per cent dextrose solution, in filter paper.	17	No anthracnose.
23	12	Prune decoction, in filter paper	17	Do.
23	100+	Wet filter paper, in damp chamber	18	Do.

As a result of the difficulties encountered and negative results secured in the endeavor to prove by laboratory methods the presence of the anthracnose fungus on commercial seed, the problem was approached in another way.

FIELD TESTS WITH DISINFECTED SEED.

Certain theoretical considerations form the basis of this method of attack. Since observation indicated that the number of original centers per acre was usually rather low, ranging perhaps from 1 to 10, and since about 2 pounds, or approximately 70,000 seeds, are used per acre, it was to be assumed on the basis of seed carriage of disease that not more than 1 to 10 seeds out of 70,000 were acting as successful bearers of the disease. In view of this possibility, the failure of the laboratory tests might be attributed in part to the relatively small numbers of seeds tested.

To obtain a test with larger numbers of seeds, recourse to field conditions was necessary. It was obvious that if the fungus could be eliminated by surface sterilization of the seed, the appearance of the disease in a new field planted with such seed should be prevented, while in a field planted with untreated seed the disease would normally be expected were the fungus present on the seed.

As to the character and effect of various seed treatments, more will be presented later. Suffice it to state here that the surfaces of seeds immersed in mercuric chlorid of a strength of 1 to 1,000 for 5 minutes were found by agar-plate tests to be sterile. With the other disinfectants employed, the effectiveness against the anthracnose fungus was assumed. Two classes of tests were made in the season of 1917, one limited to a few fields at Madison, the other being more extensive.

At Madison a mixture of 1916 Ohio seed previously tested with a new consignment from the same source was tested in four well-separated one-half acre fields on soil not previously in cucumbers or melons. In one field untreated seed was planted, while in the others seed treated as shown in Table VII was used. By planting double

the usual number of rows, twice the ordinary quantity, or about 2 pounds of seed, was planted in each field. This allowed a test of about 70,000 seeds per field.

TABLE VII.—*Effect of seed disinfection upon the occurrence of anthracnose in cucumber fields at Madison, Wis., in 1917.*

Name of field.	Area.	Subdivision.	Seed treatment.	Results.	
				On Aug. 13.	On Sept. 8.
McKenna	One-quarter acre.	West half	2 per cent formaldehyde,[1] 10 minutes.	No anthracnose.	No anthracnose.
	do	East half	4 per cent formaldehyde,[1] 5 minutes.	do	Do.
Brittenbach	do	West half	0.5 per cent $CuSO_4$, 10 minutes.	do	Do.
	do	East half	1 per cent $CuSO_4$, 5 minutes.	do	Do.
Tobacco	do	North half	Hot water, 52° C., 10 minutes.	do	Do.
	do	South half	1915 seed, untreated	do	Do.
Isom	One-half acre		1916 seed, untreated	1 plant diseased	2 centers of anthracnose.

[1] 40 per cent solution.

Until August 1 careful inspection of all of these fields was made twice each week. A special effort was made to detect an original diseased plant in the Isom field before the thinning and the elimination of the interplanted rows, but no success was attained. Unfortunately, the first anthracnose did not appear until a month after its normal date of appearance, and a complication is afforded by the earlier appearance of anthracnose in field 1 as a result of overwintering in the soil. While the Isom field was a considerable distance from field 1, there is a possibility, even though very slight, of the introduction of the disease by insects, pickers, or cultural operations. Since, however, the other fields were equally exposed to this type of infection, it is significant that anthracnose appeared only in the field planted with 1916 untreated seed. This is taken as a rather convincing indication but not conclusive proof of disease introduction with the seed.

Assuming that anthracnose was present in the 1915 seed, its failure to appear in the south half of the tobacco field has some significance. It suggests that possibly the fungus does not survive the eighteen months' desiccation.

For the more extensive field tests arrangements were made with a pickling company whereby all of the seed distributed to its growers in one district was to be previously treated, while in a neighboring district similar seed, untreated, was to be used. This trial was made in triplicate, one trial in each of the three States of Indiana, Wisconsin, and Michigan. Both Ohio seed and Iowa seed were thus tested; the former in Indiana and Wisconsin, the latter in Michigan. For the disinfection of all of this seed the treatment of proved effec-

tiveness and safety was used. This consisted of immersion in mercuric chlorid, 1 to 1,000, for 5 minutes, followed by 15 minutes' washing in running water. This bulk seed treatment, involving the handling of 400 pounds of seed, was done at Madison. By this cooperative method very large amounts of seed were tested in separate districts under a variety of conditions. As in the tests at Madison, these tests aimed not only at a proof of the theory of the seed carriage of disease but at the same time aimed at disease control, as will be noted later.

In Michigan anthracnose occurred in neither the 34 fields planted with untreated seed nor the 42 planted with treated seed, so it is obvious that there was no anthracnose in the Iowa seed. The results obtained in Indiana and Wisconsin are briefly summarized in Table VIII. It is to be noted that due allowance has been made for the complicating factor of possible overwintering in the soil.

TABLE VIII.—*Effect of seed treatment upon the occurrence of cucumber anthracnose, as shown by cooperative field tests in Indiana and Wisconsin, season of 1917.*

State.	Effect of seed treatment.						Overwintering.			
	Seed.	Number of fields.	Diseased plants.		Anthracnose not due to 1916 crop.[1]		1916 crop, same soil.		Anthracnose due to 1916 crop.[2]	
			Number.	Percentage.	Number.	Percentage.	Number.	Percentage of diseased fields.	Number.	Percentage of diseased fields.
Indiana	Untreated	30	15	50	4	13.3	9	60	11	73.3
	Treated	42	7	16.6	0	0	4	57.1	7	100
Wisconsin	Untreated	43	2	4.7	1	2.3	0	0	1	50
	Treated	33	4	12.1	1	3.3	2	50	3	75
Total	Untreated	73	17	23.3	5	6.8	9	52.9	12	70.6
	Treated	75	11	14.6	1	1.3	6	54.5	10	90.9
Gain due to treatment				8.7		5.5				

[1] 1916 crop not on same or neighboring field. [2] 1916 crop on same or neighboring field.

The results show that there was apparently very little anthracnose in the Ohio seed, since most of the cases of anthracnose occurrence may be attributed to overwintering. Among the very few cases in which anthracnose could not be due to the previous crop, the Wisconsin trials are inconclusive, while the Indiana tests indicate that anthracnose may have been introduced with the seed.

From the standpoint of the introduction of the disease with the seed, all of these seed-disinfection tests, while inconclusive, indicate that anthracnose may be carried with the seed, and they show the need of further tests of this nature.

OVERWINTERING.

Previous references have been made to the subject of overwintering, and it now remains to present the evidence relative to this phase of the problem. Sheldon (46, pp. 127–137) noted that anthracnose of watermelons was more severe where the crop was grown on the same ground or near the same ground used the previous year. Taubenhaus (51) made similar observations in Delaware and states that a 6-year rotation is thus necessitated.

In the course of the present work, much observational evidence has been accumulated during disease-survey trips, in which, however, only the statements of growers were available regarding the previous history of the fields. Concerning the 17 fields in which anthracnose was found near Sparta, Wis., in 1915, it was learned that in the case of 5 of these, cucumbers were grown on the same soil in 1914 and that in the case of 3 others the 1914 crop was adjacent to the 1915 field. The coldframe field near Norfolk, Va., previously mentioned, where anthracnose was epidemic in 1917, was in cucumbers in 1916 and the disease was present that year as well.

At Madison, Wis., anthracnose was prevalent in fields 1 and 2 in 1916. In field 1 a late epidemic left numerous diseased fruits as well as vines, which were plowed under late in the fall. In 1917, cucumbers were again planted in these fields, as well as the four mentioned in connection with the seed treatments. Field 2 was planted in part with seed treated with mercuric chlorid and in part with hand-thrashed seed from cucumbers free from anthracnose. Seed treated with mercuric chlorid was employed for all of field 1 except for one set of plats used to test out a variety of seed treatments. Among the latter were untreated control rows, but these were planted with Iowa seed which was proved to be free from anthracnose by the Michigan field trials previously described. The introduction of anthracnose into either of these fields with the seed is therefore unlikely.

In field 2 no anthracnose developed in 1917. In field 1, where much more diseased material was plowed under in 1916, anthracnose appeared, not in "original centers," but as rather scattered infected plants in at least three areas where diseased material was known to have been abundant the year before. No anthracnose developed in the three fields where treated seed was planted in soil not previously in cucumbers. The recurrence of anthracnose in field 1 under these conditions is taken as rather convincing evidence of overwintering in the soil.

In the course of inspection of the fields in the cooperative seed-treatment tests it became further apparent that the disease overwinters in the soil. It has been indicated in Table VIII that all of the outbreaks of anthracnose among the Indiana fields planted with

treated seed and in three out of the four Wisconsin fields in which the disease occurred might be attributed to overwintering. By this it was meant that the previous crop was grown on the same or a neighboring field. Disease conditions in the 1916 crop were in most cases unknown, so it can only be assumed that anthracnose was or at least might have been present. Since treated seed was used, introduction by that means was not at all probable. Of the seven diseased fields in Indiana, four were in cucumbers in 1916 and the other three were adjacent or near the field used in 1916. Of the four diseased fields in Wisconsin, two were in cucumbers in 1916, and in one of these anthracnose had occurred that year. Of the remaining two fields, one was not far from the 1916 field in which anthracnose had been very prevalent. By chance, the owner planted in 1917 one row of treated seed across this field used the previous year. Anthracnose was abundant in this row, while in the 1917 field but one diseased plant was noted.

The evidence, as summed up in Table VIII for the 75 fields where treated seed was used, shows that 6, or 54.5 per cent, of the 11 diseased fields were on the same soil used in 1916 and 4 more were adjacent to the 1916 field, making a total of 10 out of 11, or 90.9 per cent, of the cases of anthracnose possibly attributable to a previous crop.

Where untreated seed was used the evidence is not quite so convincing, but it is rather striking that in Indiana cucumbers had been grown in 1916 in 9 of the 15 infested fields and that two others were adjacent to the field used in 1916. Thus 73.3 per cent of these cases may have been due to a previous crop.

Another type of evidence is also afforded by the above tests. Of the 42 fields in Indiana planted with treated seed, 4 were in cucumbers in 1916, and anthracnose occurred in all of these in 1917; 4 more were adjacent to the 1916 field, and anthracnose occurred in 3 of these. Similarly, of the 33 fields in Wisconsin planted with treated seed, 3 were in cucumbers in 1916, and in 2 of these anthracnose was found in 1917. Out of a total of 75 fields planted with treated seed, 7 were on the same soil used in 1916, and anthracnose occurred in 6 of these, or in 85 per cent of the cases where rotation was not practiced. In 10 fields adjacent to the 1916 sites 4 showed the disease. Likewise, out of 73 fields planted with untreated seed, 14 were on the same sites used in 1916, and in 9 of these, or 64 per cent, anthracnose was found in 1917. These results are briefly summarized in Table IX.

Such observational evidence, especially where the treated seed was used on fields in which the disease was present the year before, practically proves that anthracnose overwinters in the soil.

Two rather anomalous cases are worthy of note. One is the failure of anthracnose to recur in field 2 at Madison in 1917. The

other is the fact that in one of the Wisconsin fields, where the disease occurred in 1914, 1915, and 1916, no anthracnose could be found in 1917 when treated seed was used.

TABLE IX.—*Relation between lack of crop rotation and the occurrence of cucumber anthracnose, season of 1917.*

Seed used.	Number of fields.	Fields used for cucumbers in 1916.		
		Number.	Anthracnose in 1917.	
			Number.	Percentage.
Untreated	73	14	9	64
Treated	75	7	6	85
Total	148	21	15	71

As to the mode of overwintering and the possibility of a perfect stage nothing definite is known. Using soil collected from a diseased field in October, Carsner [1] secured seedling infection in February. He also secured seedling infection in sterile soil with which were mixed chopped-up diseased vines previously kept in storage for five months. Potebnia (38, p. 82) left diseased host parts out of doors over winter but failed to find an ascus stage.

In the fall of 1916 diseased vines and fruits of various hosts were placed in wire cages and left on the ground in the garden over winter. On April 11, 1917, 14 samples of soil were taken in sterile pots from under these cages. Treated seed was planted in these pots, but no anthracnose appeared on the seedlings. Examination of some of the overwintered material in the cages on March 29 failed to show the presence of spores, and no development of the anthracnose fungus occurred under damp chamber conditions. More work should be done along this line.[2]

The present status of the problem is, then, that field observations and tests prove that the fungus overwinters in the field, although the exact mode of this overwintering is unknown.

CONTROL.

Consideration of the control of anthracnose may be conveniently divided, so far as this work is concerned, into two categories, spraying and seed treatment combined with crop rotation.

[1] Carsner, E. Op. cit.

[2] In the fall of 1917 diseased cucumber vines were buried in a small flower garden in Lansing, Mich., where anthracnose had never been present. In the summer of 1918 treated cucumber seed was planted in this spot. On August 4, 37 out of 58 plants were diseased with anthracnose, many damping-off with the disease. This proves that the fungus overwinters in the old diseased vines in the soil. Furthermore, treated 1916 seed was planted in 1918 in a 2-acre portion of the Michigan seed field which had borne a badly diseased crop in 1917. Anthracnose made its appearance at numerous and scattered points in this field in 1918, thus further proving that the fungus overwinters in the soil.

As to resistant varieties, it should be mentioned that among watermelons the citron is somewhat resistant, while practically all varieties of cucumber and muskmelon tested are susceptible.

SPRAYING.

As was pointed out in the historical account, many workers have held Bordeaux mixture to be an effective control for this disease, and the same opinion seems to be rather widely held at the present time.

At Princeton in 1915 plat tests were made of a variety of commercial sprays and of various Bordeaux formulæ. None of the applications seemed to control anthracnose, since it spread consistently through the sprayed rows.

In 1916 at Madison block tests were made with Bordeaux mixture (3–6–50 formula plus soap) and row tests with Pyrox and other strengths of Bordeaux mixture. Anthracnose spread through the sprayed rows as well as the controls, and neither observational nor yield-record evidence indicated that any of the spray applications controlled the disease. Its severity was, however, somewhat checked by the sprays, since it was very serious in only 5 out of 17 sprayed rows, as compared with 7 out of 11 unsprayed controls.

To gain some insight into the effect of the presence of the spray upon leaf infection, a series of atomizer inoculations were made on August 13 upon the upper surfaces of certain leaves and the lower surfaces of other leaves among the sprayed rows. Well-sprayed leaves were chosen. The results of these inoculations are presented in Table X.

TABLE X.—*Results of atomizer inoculation of sprayed cucumber leaves at Madison, Wis., in 1916.*

Spray treatment.	Number of leaves.	Surface inoculated.	Results, Aug. 24.
None	1	Both	?
Do	10	Upper	−(8 located. No infection).
Do	10	Lower	?
Bordeaux mixture, 3–6–50 formula	10	Upper	−(9 located).
Do	10	Lower	+(9 located; all infected).
Do	36	Upper	−
Do	25	Lower	+(7 typically infected).
Bordeaux mixture, 2–4–50 formula	25	Upper	−
Do	25	Lower	+(6 typically infected).
Bordeaux mixture, 4–6–50 formula	25	Upper	−
Do	25	Lower	+(4 typically infected).
Pyrox	25	Upper	−
Do	25	Lower	+(9 typically infected).

While natural infection interfered to some extent with these tests, no unquestionable atomizer infection occurred in any case where the upper epidermis of sprayed leaves was inoculated, while in all cases some typical atomizer infection was secured where the lower epidermis of sprayed leaves was inoculated. Of these leaves, only the upper epi-

dermis was covered with the spray. These results indicate that an actual coating of spray material prevents infection through the upper epidermis, but that infection may readily occur through the unprotected lower epidermis.

In practice, it is impossible to coat the lower surfaces of the leaves, and a spray is therefore only one-half efficient as a protection. Furthermore, the rapid growth of runners constantly exposes unprotected tissue.

For the partial protection afforded, growers of melons, field cucumbers, and seed crops can probably spray with profit. The fruits in these cases are longer exposed to infection and the economic difficulties are negligible. But in the cucumber-pickle industry, where the crop is grown in widely scattered small patches, usually among the less prosperous farmers, the practical difficulties in any spraying program become insurmountable.

SEED DISINFECTION AND CROP ROTATION.

Since it has been shown that the disease overwinters in the soil, it goes without saying that a rotation of crops should be practiced and that clean soil should be considered a prime requisite. The site chosen should be well removed from any field in which the disease was present the preceding year.

Given a clean soil, the problem of disease prevention depends largely upon the validity of the seed-carriage hypothesis previously presented. If this hypothesis is correct, prevention depends upon the development of a harmless, effective, and practicable seed treatment or upon the availability of disease-free seed. Regarding the availability of disease-free seed, anthracnose seems to be prevalent in eastern seed farms, but not in Colorado. Although the disease was reported from that State in 1917, Colorado-grown seed should be comparatively free from anthracnose.

Concerning the effect of seed treatment upon germination, much remains to be done. Injury has resulted from hot water and formaldehyde. Carsner found that hot water, 52° C. for 10 minutes, was harmless. Field tests in 1917 indicate the safety of all of the treatments except that of formaldehyde in Table VII. The 4 per cent formaldehye[1] for 5 minutes caused great damage and the 2 per cent solution for 10 minutes caused rolling of the cotyledons. Mercuric chlorid, 1 to 1,000, for 5 minutes appears to be harmless except that in some cases a very slight retardation after germination can be detected. Copper sulphate is somewhat objectionable because of the bluish stain left on the seed.

While the 1917 experimental field tests at Madison were inconclusive because of the late appearance of disease in the control field, it

[1] 40 per cent. solution.

is of interest to note that the 1915 seed was apparently as free from disease as treated seed. All of the treatments appear to have been effective in these field tests.

The effectiveness of formaldehyde, 1 per cent for 20 minutes, and of mercuric chlorid, 1 to 1,000, for 5 minutes, was tested in the laboratory by avoiding contamination after sterilization and planting the seed in culture media. One test was made on seed previously inoculated. In all cases, however, the effect upon the normal surface flora of the seed was used as a criterion. The results of these tests are presented in Table XI.

TABLE XI.—*Laboratory tests of the treatment of cucumber seeds with mercuric chlorid and formaldehyde for the control of anthracnose.*

Seed used.	Date, 1917.	Method.	Mercuric chlorid.		Formaldehyde.	
			Number tested.	Results.	Number tested.	Results.
Ohio	Apr. 9	2 per cent dextrose agar, in plate.			20	10 sterile; 1 with fungus; 9 with bacteria.
Inoculated on Mar. 13, 1917.	do	do	20	All sterile	20	4 sterile; 4 with fungi; 12 with bacteria; no anthracnose.
Do	May 13	do	30	All sterile except one; anthracnose on cotyledon.	40	None sterile; 7 with fungi; no anthracnose.
Do		Bouillon tubes	10	9 sterile; 1 developed a fungus.		
Do		Nutrient agar	16	All sterile		

These results show that the mercuric-chlorid treatment is more reliable than the formaldehyde in absolute effectiveness. The very interesting case in which 1 out of the 136 inoculated seeds yielded a diseased seedling deserves some comment. The age and location of the lesion indicated that it had resulted from a recent spore infection rather than an earlier micropylar invasion. This would mean that the spore had not only escaped the germicide but had also survived two months of desiccation. It was probably lodged in the micropyle and protected from the germicide by an air bubble.

Owing in part, no doubt, to organic matter on the seed, there is a marked decrease in the strength of mercuric-chlorid solutions after seed is immersed therein. Six liters of seed decreased the concentration of mercuric chlorid in eight liters of the germicide by almost one-half. This means that the solution can be safely used only once or twice.

The effectiveness of a 5-minute immersion in weaker solutions has been tested by the agar-plate method. Using about 100 seeds for each series, the following percentages of sterile seeds were obtained:

Mercuric chlorid, 1 to 1,500..... 99 per cent sterile.
Mercuric chlorid, 1 to 2,000..... 94 per cent sterile.
Mercuric chlorid, 1 to 3,000..... 97 per cent sterile.

These results indicate that lower concentrations are fairly effective. For practical use, however, the standard strength of 1 to 1,000 is preferable.

In the large-scale field tests summarized in Table VIII, the determination of the value of seed disinfection as a control measure is complicated by the relatively few cases of anthracnose occurrence in which there was no possibility of soil infestation. Disregarding this contingency, it is rather striking that the disease was present in only 14.6 per cent of the fields planted with treated seed as compared with 23.3 per cent of the controls. This indicates a prevention of disease in 8.7 per cent of the fields, or, on a comparative basis, prevention of 37 per cent of the cases that would normally have occurred. Eliminating all cases possibly attributable to overwintering, we find the disease present in 1.3 per cent of the fields planted with treated seed as compared with 6.8 per cent of the controls, indicating a net gain of disease prevention in 5.5 per cent of the fields, or, on the comparative basis, a prevention of 80 per cent of the outbreaks.

In conclusion, it may be said that a successful control of anthracnose may quite possibly be secured by the use of disease-free seed in clean soil.

SUMMARY.

Anthracnose of cucurbits is caused by the fungus *Colletotrichum lagenarium* (Pass.) Ell. and Hals.

The hosts are limited to the family Cucurbitaceæ. Those of economic importance are the cucumber, muskmelon, and watermelon. The disease is common among Lagenaria gourds. The following cucurbits are added to the list of hosts: *Benincasa cerifera*, *Trichosanthes colubrina*, *Cucumis dipsaceus*, *C. melo* var. *dudaim*, and *C. melo* var. *flexuosus*. Fruit infection occurred in *Cucumis anguria* and *C. anguria* var. *grossulariae*. Anthracnose was not found as a vine disease in the genus Cucurbita, which includes squash, pumpkin, and certain gourds. Beans are not susceptible.

This disease was first noticed in 1867 in Italy among Lagenaria gourds. Later, it appeared on melons in France, and it now occurs throughout Europe and the eastern United States wherever the hosts are grown.

Serious losses are caused in the watermelon-growing industry of this country and among cucumbers grown for slicing purposes. Since the disease is more serious on crops in which the fruits are long exposed to infection, the losses in the cucumber-pickle crop are not as great.

Leaves, stems, and fruits are attacked. Leaves and even whole runners and plants may be killed, and fruits may be blemished or become malformed. The lesions increase rather indefinitely in size.

Fruiting bodies, or acervuli, are conspicuous on fruit lesions, occur in abundance on stem and petiole lesions, and are less abundant on leaf lesions.

The fungus grows and sporulates readily in culture and is not exacting as to nutrients. Under optimum conditions the life cycle may be completed in four days.

As to essential elements, iron and sulphur are not needed in detectable concentrations, and only very minute amounts of magnesium are necessary. As sources of carbon the more complex carbohydrates seem to be more suitable. Corn starch and xylan were used to good advantage, and fair growth occurred on cellulose.

The fungus is quite sensitive to copper sulphate. Growth was prevented by a concentration of molecular÷2,000 and was retarded in molecular÷64,000.

Spore germination is favored by the presence of a nutrient and by a plentiful supply of oxygen. The optimum temperature for spore germination lies between 22° and 27° C.; the minimum is about 4° C. Guttation water from cucumber leaves is apparently not toxic to spores. There is evidence that spores may germinate in moisture condensed on the lower sides of watermelon fruits in the field.

Thick-walled egg-shaped appressoria are normally formed by germinating spores. These appressoria are usually in contact with a solid substratum. An abundant oxygen supply favors their formation. Appressoria were not formed at 7° C. and below.

Spores germinating upon a host normally form appressoria closely adherent to the cuticle. Host penetration occurs directly through the leaf cuticle, not through a stoma. The penetration tube issues from a small pore in the under side of the appressorium.

The mycelium is intracellular. Invaded host cells become collapsed and stain deeply. There seems to be a previous stimulation of nuclear and cell division.

In a field the disease usually appears first in isolated "original centers" of one or two infected plants each. Marked spread of the disease from these centers follows rainy periods, particularly, it would seem, when the temperatures are not too far above or below 75° F., the optimum for this fungus. The disease ordinarily becomes epiphytotic only late in the life of the host crop.

The principal agencies of dissemination in the field are rain and surface drainage water. The spore masses disperse readily in water. During rains the spores are washed to the soil and thence splashed upon the leaves. Centers of infection are thus enlarged. Extensive spread from such centers is usually in the direction of the slope and is accomplished by surface drainage during heavy rains. The fungus

has been isolated from soil under diseased plants and from surface drainage water after rains.

The disease is especially destructive in certain fields where artificial overhead watering is practiced. Convincing evidence of hand dissemination by workmen during the process of culling watermelon fields has been secured.

The mode of origin of the disease in new fields in original centers and its appearance in only the fields planted with seed from certain sources suggest that the fungus is introduced with the seed.

Anthracnose has been found prevalent in seed fields on the fruits. The process of seed extraction affords ample opportunity for wholesale surface contamination of seed. The cellulose "hairs" on the seed coat would afford lodgment and protection to spores. Fragments of tissue from fruit lesions have been found among and adherent to seeds. No evidence of the presence of dormant mycelium within seeds removed from beneath deep fruit lesions has been secured.

Numerous laboratory and greenhouse tests have failed to prove the presence of infectious material on commercial seed. With inoculated seed, diseased seedlings result.

Extensive field tests with treated and untreated seed, while inconclusive, indicate that the fungus is carried with the seed.

Convincing evidence that the fungus overwinters in the field has been accumulated. The disease recurs annually in certain localities and not in others. In experimental fields planted with treated seed, the disease reappeared in those which bore a diseased crop the previous year. In extensive field tests with treated seed, 90 per cent of the cases of anthracnose may possibly be thus explained. It has been proved that the fungus overwinters in diseased-vine débris buried in soil.

Bordeaux sprays check but do not prevent the spread of the fungus. The lower epidermis of a sprayed leaf is unprotected. Spraying may be advisable in the melon, slicing-cucumber, and seed-cucumber crops, but is not practicable in the cucumber-pickle growing industry.

It is believed that surface disinfection of the seed will eliminate the infectious material. For this purpose, immersion in mercuric chlorid, 1 to 1,000, for 5 minutes has been found effective and practically noninjurious. Disease-free seed may also be secured from disease-free seed fields, and possibly by the use of old seed.

The use of disease-free seed and a proper crop rotation to insure clean soil are recommended as control measures.

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